

# THE PLANT DISEASE REPORTER

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Paul R. Miller

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PLANT DISEASE REPORTER  
Mycology and Plant Disease Reporting Section  
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THE ACTION OF ESSENTIAL OILS ON PHYTOPATHOGENIC FUNGI<sup>1</sup>Jasper C. Maruzzella and Jerry Balter<sup>2</sup>

## Abstract

The *in vitro* activity of 119 essential oils was tested against 12 phytopathogenic fungi using the filter paper disk method. Eighty-four percent of the oils were found to produce antifungal activity on at least 2 of the 12 test organisms. The greatest zones of inhibition were produced by oil of onion, garlic, thyme white, thyme red, *origanum rectified* (water white), *origanum bay*, *lemongrass*, sweet birch (northern), and Bois de Rose. Eighty percent of the essential oils were found to kill *Claviceps purpurea* (the most vulnerable organism) while 58 percent of the oils were active against *Alternaria tenuis* (the least susceptible organism). It is suggested that perhaps it may be practical to use certain essential oils in plant disease control.

## INTRODUCTION

Investigations on the antimicrobial activity of essential oils has been directed almost exclusively toward human and animal pathogenic microorganisms (1, 2, 6, 8, 9, 10, 12). The data that has accumulated on the action of essential oils or fixed oils on phytopathogenic microorganisms is rather scanty (3, 5, 7). More extensive investigations of these plant products might prove these to be valuable potential aids in plant disease control. In order to obtain data that would be useful in subsequent *in vivo* studies, this *in vitro* examination of essential oils of phytopathogenic fungi was undertaken.

## MATERIALS AND METHODS

The antifungal activity of essential oils was studied by observing their effects on growing cultures of a variety of phytopathogenic fungi. The fungi used were: *Fusarium oxysporum* f. *conglutinans* (ATCC 9990), *Fusarium oxysporum* f. *lycopersici* (ATCC 9848), *Ceratostomella ulmi* (Coll. No. 50525), *Myrothecium verrucaria* (ATCC 9095), *Ustilago avenae* (strain 3 U. A. 38), *Verticillium albo-atrum* (NRRL 1204), *Claviceps purpurea* (NRRL 1270), *Botrytis allii* (NRRL 2502), *Gibberella fujikuroi* (NRRL 22844), *Diplodia maydis* (NRRL 2282), *Cladosporium fulvum* (ATCC 10391), and *Alternaria tenuis* (ATCC 6663). The method of Vincent and Vincent (13) was employed for detecting the presence or absence of antifungal activity. In this filter paper disk diffusion plate method sterile disks (6.35 mm diameter) were thoroughly saturated with the oil to be tested. The saturated disks were placed on the surface of Sabouraud's maltose agar in plates which had been previously seeded with 1 ml of fungal broth culture. All dishes were conducted in triplicate with one disk per dish. In some instances the essential oil on the disk produced completely clear dishes (100 mm diameter). When this occurred larger dishes (150 mm diameter) were used in repeating the experiment. Here also some essential oils cleared the large dishes. When this occurred such zones of inhibition were recorded as 100 mm. All of the organisms were cultivated in Sabouraud's maltose broth for 1 week at room temperature. The dishes were incubated for 4 days at room temperature except those containing *M. verrucaria* and *U. avenae* which were incubated for 1 week at room temperature. The presence of zones of inhibition surrounding the saturated paper disks indicated antifungal activity and were recorded with a metric ruler with the aid of an illuminated Quebec colony counter.

## RESULTS AND DISCUSSION

Of the 119 essential oils tested 100 (84 percent) were found to possess antifungal activity against at least 2 of the 12 test organisms. Table 1 shows the 100 essential oils with zones of inhibition produced against each fungus. It is interesting to note that of the 100 oils listed in Table 1, 51 showed a wide spectrum of activity on all fungi tested. The greatest zones of inhibition were produced by oil of: onion, garlic, thyme white, thyme red, *origanum rectified*

<sup>1</sup>The authors are indebted to Fritzsche Brothers, Inc. New York City for their generous supply of essential oils used in this study.

<sup>2</sup>Associate Professor of Biology and graduate student, Department of Biology, Long Island University, Brooklyn, New York. This investigation was supported by the Research Fund of Long Island University.

Table 1. Inhibitory activity of essential oils.<sup>a</sup>

Oils	<i>F. oxysporum</i> <i>f. conglutinans</i>	<i>F. oxysporum</i> <i>f. lycopersici</i>	<i>C. ulmi</i>	<i>M. verrucaria</i>	<i>U. avenae</i>	<i>V. albo-atrum</i>	<i>C. purpurea</i>	<i>B. allii</i>	<i>G. fujikuroi</i>	<i>D. maydis</i>	<i>C. fulvum</i>	<i>A. tenuis</i>
Abies alba (from cones)	0	0	2	0	7	4	8	0	0	0	3	0
Amyris	0	0	0	5	0	0	0	0	0	2	0	3
Angelica root	0	0	4	0	3	3	2	2	0	0	0	0
Anise, USP	5	6	20	20	100	10	16	3	5	4	13	10
Balsam Peru	10	3	7	4	15	12	10	0	2	2	2	3
Basil, sweet	5	8	15	10	5	4	11	4	0	0	20	20
Bay, NF	22	30	25	27	32	30	25	15	23	20	35	22
Bergamot, NF	2	0	1	5	8	3	3	0	3	0	2	0
Birch tar, rectified	9	15	16	16	25	16	15	18	15	18	11	12
Bois de Rose	15	12	10	40	24	14	16	40	10	5	40	57
Cade, rectified	10	5	5	9	18	18	12	12	7	10	12	8
Cajuput	2	4	6	6	12	5	10	5	5	2	2	2
Calamus	2	2	3	5	12	3	7	2	2	5	4	5
Camphor, sassafrassy	10	5	7	5	34	15	10	15	5	11	12	12
Camphor, white	3	0	0	0	8	7	8	1	3	0	0	0
Caraway, NF	6	15	37	35	10	15	24	12	20	11	34	12
Cardamom, NF	0	0	3	25	10	4	3	3	4	0	3	5
Cascarilla	3	3	3	3	6	5	3	8	5	0	1	1
Cassia, USP	15	15	20	10	35	30	24	15	12	10	20	16
Cedar leaf	0	0	5	0	12	5	8	0	3	0	3	0
Celery seed	6	2	4	10	15	5	0	2	2	5	5	3
Chamomile, German	0	0	0	2	0	0	17	2	0	0	0	0
Chenopodium	14	30	27	10	44	25	23	7	20	26	12	12
Cherry laurel	7	11	5	10	0	10	55	18	8	55	0	12
Cinnamon, Ceylon	20	15	25	15	30	21	22	35	15	17	13	15
Citronella, Formosan	3	8	10	35	12	15	12	30	15	23	50	22
Clove, USP	11	13	15	16	28	14	18	20	10	18	25	20
Clove leaf, rectified	18	15	16	20	25	5	12	8	16	20	14	14
Cognac, green	0	0	2	5	7	5	5	1	0	0	3	0
Coriander, USP	5	8	6	25	30	20	10	25	11	10	65	28
Cumin	24	18	20	30	10	35	25	30	16	15	25	25
Curacao peel	0	0	2	0	0	0	3	0	0	0	0	0
Dill weed	15	30	20	16	12	2	20	30	10	17	9	5
Estragon	30	7	25	7	23	2	10	100	5	25	12	16
Eucalyptus, rectified	0	2	2	0	7	7	6	0	2	0	3	0
Fennel, USP	9	10	20	28	25	20	11	6	10	2	35	22
Galbanum	0	0	0	2	0	3	3	0	0	0	0	0
Garlic, imported	2	25	30	100	17	15	100	100	50	30	100	30
Geranium, Algerian	5	3	5	10	25	11	27	6	3	2	10	5
Hemlock	0	0	0	0	3	5	2	0	0	0	0	0

Table 1 (continued)

Oils	<i>F. oxysporum</i> <i>f. conglutinans</i>	<i>F. oxysporum</i> <i>f. lycopersici</i>	<i>C. ulmi</i>	<i>M. verrucaria</i>	<i>U. avenae</i>	<i>V. albo-atrum</i>	<i>C. purpurea</i>	<i>B. allii</i>	<i>G. fujikuroi</i>	<i>D. maydis</i>	<i>C. fulvum</i>	<i>A. tenuis</i>
Labdanum	11	6	12	7	10	18	11	13	5	13	3	5
Laurel leaf	6	5	5	5	8	12	11	5	7	0	5	8
Lavandin	2	2	2	16	30	10	10	4	3	0	3	0
Lavender, USP	3	4	4	15	21	15	6	4	5	3	3	8
Lemon, cold pressed												
California	0	0	0	0	3	3	10	0	0	0	0	0
Lemon, hand pressed												
Italian	0	0	3	0	5	2	6	0	3	0	0	0
Lemongrass, rectified	15	30	25	43	28	30	15	28	20	20	30	15
Lime, expressed	2	0	2	2	3	5	5	0	2	0	2	0
Lime, distilled	4	0	3	0	3	10	6	3	3	0	1	0
Linaloewood	5	9	10	32	18	3	12	18	12	10	35	12
Lovage	2	1	2	5	4	5	4	3	2	3	3	2
Mace	8	4	12	4	19	14	22	6	9	0	5	5
Mandarin, Italian	1	0	0	0	2	3	3	0	0	0	0	0
Majoram, sweet	10	14	25	20	25	20	17	20	20	18	10	12
Mountain laurel	15	9	18	20	11	27	30	20	12	25	17	20
Neroli												
Bigarade												
Petale, NF	13	13	12	18	16	5	20	8	4	0	15	12
Nutmeg, East Indian, USP	20	8	14	22	35	25	20	12	13	20	8	10
Nutmeg, West Indian, USP	5	0	12	16	20	0	15	4	10	4	2	3
Ocotea												
Cymbarum	13	5	5	14	10	0	25	10	5	10	10	13
Olibanum	0	0	3	4	6	10	10	0	0	0	1	0
Onion	30	20	100	100	100	60	100	35	5	100	100	100
Opopanax	0	0	0	0	3	0	2	0	0	0	0	0
Orange, bitter	0	0	0	0	5	4	4	0	2	0	0	0
Origanum	25	21	27	35	27	30	25	47	25	32	15	30
Origanum, rectified, water white	35	20	26	50	31	28	30	30	20	30	40	43
Palmarosa	3	5	10	11	37	11	8	15	10	0	9	5
Parsley seed	0	0	2	5	9	2	4	5	1	3	2	0
Patchouly, Singapore	3	0	0	2	0	0	0	0	0	0	0	2
Pennyroyal, imported	15	12	28	35	8	3	15	30	13	35	40	48

Table 1 (concluded)

Oils	<i>F. oxysporum</i> <i>f. conglutinans</i>	<i>F. oxysporum</i> <i>f. lycopersici</i>	<i>C. ulmi</i>	<i>M. verrucaria</i>	<i>U. avenae</i>	<i>V. albo-atrum</i>	<i>C. purpurea</i>	<i>B. allii</i>	<i>G. fujikuroi</i>	<i>D. maydis</i>	<i>C. fulvum</i>	<i>A. tenuis</i>
Peppermint, rectified, USP	3	4	23	20	13	25	6	17	5	6	23	20
Peppermint, natural	4	3	22	35	15	6	10	45	6	18	60	30
Petitgrain, Paraguay	3	2	2	28	32	4	5	12	7	3	5	5
Pimenta (from berries)	14	18	20	18	30	15	18	20	15	20	15	16
Pimenta leaf	14	18	20	10	30	15	10	21	14	20	20	17
Pinus sylvestris	0	0	0	0	4	0	3	0	0	0	0	0
Rose, Kazanlik, USP	4	3	7	15	25	9	5	10	6	0	10	5
Rosemary, NF	3	0	2	1	6	7	8	0	7	0	2	0
Rosemary acetylated	2	3	4	0	16	8	7	0	3	0	2	0
Rue	1	2	5	10	20	3	7	2	3	3	4	0
Sage, Clary	5	2	2	14	20	7	5	5	3	5	5	5
Sage, Dalmatian	2	0	3	2	15	10	8	5	5	0	3	0
Sassafras, NF	8	6	10	40	15	14	15	5	17	25	10	15
Savin	3	1	15	6	5	3	2	0	3	0	2	2
Snake Root, Canada	6	2	6	19	23	7	7	5	4	6	6	5
Spearmint, NF	10	30	35	30	30	23	16	8	10	25	18	20
Spike lavender	3	6	12	30	10	15	20	15	12	0	5	5
Spike lavender acetylated	0	0	0	2	2	2	3	0	0	0	0	0
Spruce	0	0	0	0	2	2	3	0	0	2	0	0
Styrax	15	13	20	12	20	16	20	16	15	13	13	12
Sweet birch northern, USP	26	18	40	35	12	30	7	10	6	30	55	25
Tansy	2	3	5	4	20	2	7	5	7	0	5	5
Tar, rectified, NF	16	23	29	25	35	20	20	30	16	20	18	14
Thyme, red, NF	32	22	22	50	38	12	40	20	27	33	20	26
Thyme, white, NF	30	34	30	60	30	15	22	65	25	40	57	44
Turpentine, rectified, NF	0	0	0	0	3	3	6	0	0	0	0	0
Valerian, Indian	8	5	5	4	20	10	14	6	8	12	5	5
Vetiver, Haiti	1	0	1	3	0	0	0	0	0	0	0	0
Wintergreen, northern, USP	7	20	25	35	15	5	13	8	15	18	27	45
Wormwood	0	0	0	27	13	0	11	8	0	15	30	7
Ylang Ylang	6	2	6	3	15	10	6	3	4	1	3	1

<sup>a</sup>Zones of inhibition in mm, measured from disk edge to zone edge.

(water white), origanum, bay, lemongrass, sweet birch (northern), and Bois de Rose. The 19 essential oils (16 percent) found to possess no antifungal activity were: Abies alba (from needles), Abies sibirica, cananga rectified, cedar wood, copaiba, cubeb, ginger, grapefruit (expressed, Florida), guaiac wood, hops, juniper twice rectified, myrrh, orange (sweet California cold pressed USP), Orris root (Florentine), pepper black, persic, Pinus pumilio, sandalwood, and tangerine. Fifty-one percent of the oils tested were active against D. maydis, 58 percent against A. tenuis, 59 percent against F. oxysporum f. lycopersici, 63 percent against B. allii, 65 percent against F. oxysporum f. conglutinans, 67 percent against G. fujikuroi, 68 percent against C. fulvum, 69 percent against M. verrucaria, 71 percent against C. ulmi, 75 percent against V. albo-atrum, 78 percent against U. avenae, and 80 percent against C. purpurea. The most effective essential oil against each test organism was of the following order (data taken from Table 1): origanum (rectified water white) on F. oxysporum f. conglutinans; thyme white on F. oxysporum f. lycopersici; garlic and onion on M. verrucaria; anise, garlic and onion on U. avenae; garlic and estragon on B. allii; onion and garlic on C. fulvum; onion on C. ulmi, V. albo-atrum, D. maydis and A. tenuis; and garlic on C. purpurea and G. fujikuroi.

Whether these powerful inhibitors of phytopathogenic fungi may be used *in vivo* in diseased plants has not been thoroughly investigated. Their toxicity is certainly a factor to be considered since some oils are toxic to human and animal tissue. It does not seem unreasonable to suggest that small amounts of certain essential oils when added to materials such as fungicidal sprays used in plant disease control might enhance the effectiveness of the preparation. Indeed it has been found that some essential oils enhance the antibacterial activity of certain antibiotics (4, 11). At present this area is being investigated.

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# A WHITE HEART ROT OF FRAXINUS CAUSED BY FOMES JOHNSONIANUS (MURR.) LOWE

Ross W. Davidson<sup>1</sup>, Frances F. Lombard<sup>1</sup>, and W. A. Campbell<sup>2</sup>

## Summary

Studies conducted at the Beltsville Laboratory show that *Fomes johnsonianus* occurs primarily on ash in certain areas, causing a white laminated rot that may appear as a white mottled rot in incipient stages.

In October 1932 extensive fruiting of a brown fungus identified by L. O. Overholts as *Fomes densus* Lloyd was collected on a large log belonging to the white ash group in Fairfax, Virginia. Cultures obtained from the sporophore were the same as those isolated by Overholts and Kaufert from ash logs in Louisiana in 1931. The cultures from Virginia and Louisiana were used by Campbell (1) in his published description of the cultural characteristics of *Fomes densus* Lloyd. In a recent monograph of the genus *Fomes*, Lowe (6) considers *F. densus* as interpreted by Overholts (8) to be *F. johnsonianus* (Murr.) Lowe.

In 1931 Kaufert (5) began a study of decay in fire damaged hardwoods in Louisiana. He spent considerable time isolating decay fungi from sporophores collected by Overholts (7). He also made numerous isolations from decay associated with fire wounds on living trees. The study of decay associated with fire wounds on hardwoods was continued in 1932 by Hepting (4). Hepting's report also included some of the Kaufert 1931 data.

Although some information was given on the fungi associated with decay, a number of the unidentified cultures from these studies were sent to the Beltsville Forest Disease Laboratory for further study. Seven of these cultures from decay in living trees were finally identified as *Fomes johnsonianus* (*F. densus*). These seven cultures were all from heart rot in living ash. Fruiting occurs on logs of infected trees after they fall over or are cut down. Table 1 lists the specimens and trees from which cultures were obtained.

Table 1. Trees and specimens from which *Fomes johnsonianus* was isolated.

Specimen or tree number	Source of culture	Host substratum	Locality	Date	Collector
<b>Specimen:</b>					
55500	Sporophore	Fraxinus (log)	Louisiana	1931	Overholts & Kaufert
50383	do.	do.	Louisiana	1931	Overholts & Kaufert
52033	do.	do.	Virginia	1932	Davidson
71531	Sporophore & Rot	do.	Ohio	1936	Lorenz
<b>Tree:</b>					
169	Rot	Fraxinus (living tree)	Louisiana	1931	Kaufert
315	do.	do.	Louisiana	1931	Kaufert
166	do.	do.	Louisiana	1932	Hepting
187	do.	do.	Louisiana	1932	Hepting
191	do.	do.	Louisiana	1932	Hepting
195	do.	do.	Louisiana	1932	Hepting
522	do.	do.	Louisiana	1932	Hepting

Overholts (8) records the fungus as occurring on *Fraxinus* and other hardwood trees. Lowe (6) states that it occurs on "Angiosperms" with the "Type of rot unknown." One of the sporophores on ash from Louisiana is associated with a white laminated rot. This is the same type of rot associated with the sporophore collected in Fairfax, Virginia (Fig. 1, B). Decay in four of the seven trees from which the fungus was isolated was recorded as a white rot; one of these was further defined as a white mottled rot. The advanced stage of the rot caused by *F.*

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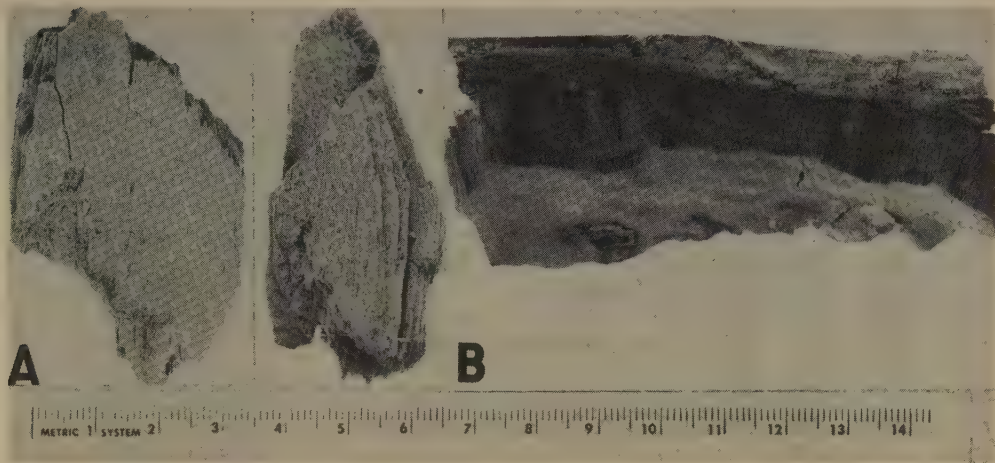


FIGURE 1. *Fomes johnsonianus* (Murr.) Lowe from white ash log (*Fraxinus* sp.). A -- White laminated decay associated with sporophore. B -- Portion of sporophore with wood attached. (Specimen number F.P. 52033, from Fairfax, Virginia)

*johnsonianus* is a white soft laminated type (Fig. 1, A) but in more incipient stages is a white mottled rot.

#### CULTURAL CHARACTERISTICS OF *F. JOHNSONIANUS*

The description given by Campbell for *F. densus* (1) is correct for this fungus. His illustration of a Petri dish culture shows its mat character on malt agar medium. The Key Pattern should be B-P-M-11 (3). There are no outstanding microscopic features but its distinguishing characteristics are the yellow, "Primuline Yellow" to "Cream Color" or "Colonial Buff," color that may occasionally be a darker yellow to brown, "Antimony Yellow," fading to a slightly raised white marginal zone 3 to 4 mm wide. The central area over the inoculum is raised and gradually thins out toward the margin of the mat. Growth and reaction on gallic and tannic acid media is given for *F. densus* by Davidson et al. (2) and shows a strong reaction characteristic of the white rot fungi.

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ELIMINATION OF VERTICILLIUM ALBO-ATRUM BY COMPOSTING COTTON GIN WASTES<sup>1</sup>E. E. Staffeldt<sup>2</sup>Abstract

Composting of cotton gin wastes was found to be effective in eliminating the viable hyphae, conidia and microsclerotia of Verticillium albo-atrum. Stem portions of naturally infected cotton were embedded in a pile without amendments and three piles containing additions of antagonistic fungi or two different types of commercial preparations. The maximum temperatures developed by the four semi-aerobic compost piles ranged from 61.7° to 68.3° C. Verticillium was not isolated on agar platings from any stems 14 days after composting.

## INTRODUCTION

The low inherent organic content of southern New Mexico soils makes desirable the regular addition of organic matter. One such source of organic matter is the waste cotton plant residues resulting from ginning operations. Much of this plant material from the cotton-growing areas of New Mexico harbors Verticillium albo-atrum. During the past few years, an increasing amount of this material was returned to the soil without prior treatment. A number of farmers began adding cotton gin trash to their fields as soon as it became available. Other farmers waited until after frost before applying these infected plant tissues to the soil. Still others used this material for cattle bedding prior to application to the fields. Regardless of the above-mentioned methods of handling this material, V. albo-atrum remained viable. Blank and Leyendecker (1) have conclusively shown that infected plant residues are excellent sources of inoculum.

## MATERIALS AND METHODS

In cooperation with studies on the composting of gin trash by the United States Cotton Ginning Laboratory at Mesilla Park, New Mexico, it was possible to conduct limited experiments on the survival of V. albo-atrum in semi-aerobic, trench-type compost piles. Cotton plant residues, obtained from a local gin, had previously been put through a hammermill. This material was watered before placing it in four identical test piles, and was watered again as it was placed in the enclosures. An enclosure consisted of a 6 x 8 x 2-foot pit (Fig. 1). The depth of the pit was extended another 2 feet above ground level by using a slatted fence supported externally by soil. A slatted, raised floor was placed at the bottom of each pit and air tunnels were provided in all four corners and center to enhance the diffusion of gases. The sides were lined with 15-pound roofing felt to assist in retaining the moisture.



FIGURE 1. Semi-aerobic trench-type compost piles included left to right, 1) Soil Biotics; 2) Fertosan; 3) fungi and 4) a non-amended check.

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Verticillium albo-atrum was isolated from every cotton stalk prior to its use in this experiment. Portions of wilt-infected cotton stalks, 6 inches in length and 1/2 inch in diameter, were attached every 6 inches to heavy wire and planted in the four compost piles. Thus, six cotton stalks were attached to each wire and the stalks distributed from the center to 2 inches from the edge of the pile. Six wires were placed in each pile, one across each quarter and two across the center of the pile. These wires were placed 2 feet from the bottom of the pit as the compost pile was being constructed.

Each of the four compost heaps consisted of a different treatment, which included: 1) Soil Biotics; 2) Fertosan; 3) fungi and 4) a non-amended check. Soil Biotics and Fertosan are commercial preparations containing microorganisms that assist in the decomposition process. Treatment Number 3, fungi, contained six fungi, found in the laboratory to be highly competitive, antagonistic or effective against normal development of V. albo-atrum.

The interior temperatures of the compost heaps were chart-recorded during the entire period by resistance thermometers. These four thermometers, one in each pile, were placed 18 inches from the outer edge of the pile and at the same depth as the cotton stalks.

## RESULTS

The recorded temperatures (Fig. 2) indicated that within 4 days a temperature over 50° C had developed in each of the four experimental enclosures. The gin trash seeded with fungi exceeded this temperature within the first 24 hours, while the same temperature was exceeded in the check pile and those seeded with Fertosan and Soil Biotics within 2, 3 and 4 days respectively. Following the early, rapid temperature rise in the fungi-seeded material, a distinct thermal decline resulted between the fourth and sixth days. From the seventh through the twelfth day, the temperature continually increased to another high of 68.3° C. The organisms originally added were apparently replaced by others more suited to the higher temperatures. Only slight fluctuations occurred from the slow temperature decline over the remainder of the 36-day period. The six fungi originally introduced into this experimental pile were not recovered in the composted material on termination of this experiment.

In the check pile the peak temperature of 68.3° C was reached on the fourth day and was followed by a temperature decrease to 58.9° C on the eighth day. The recorded temperature over the remaining period appeared cyclic, with the low temperature of the cycle occurring on or near every sixth day (8, 14, 20, 26 and 33).

The compost heap seeded with Soil Biotics developed a peak temperature of 66.7° C on the seventh day and a gradual temperature decline resulted during the remainder of the experiment.

Microbial activity in the compost heap seeded with Fertosan was not as accentuated in the high and low temperatures as were the other piles. A temperature of 60° C was developed by the fourth day and remained at this level until the fourteenth day. The peak temperature of 65° was developed on the nineteenth day, and this was followed by 13 additional days near 60°. Finally a temperature decrease was indicated on the thirty-fourth day of the experiment.

Verticillium albo-atrum was isolated from every cotton stalk prior to its use in this experiment. Two wires and the attached cotton stem portions were removed from each pile after 14 days. Three pieces of stalk tissue were planted on agar plates from each of the 48 cotton stalk portions removed. Thus 144 pieces of tissue were examined and growth of Verticillium from these tissues was not observed. At the same time nine isolations were made from three portions of cotton stalks that had been maintained under laboratory conditions. In every case, V. albo-atrum was found growing from this material maintained as a check. At the termination of the experiment the remaining cotton stalk portions were removed. Verticillium albo-atrum was not observed growing from any of the 288 pieces of tissue examined, by planting on agar plates.

## DISCUSSION

Nelson and Wilhelm (2) reported that a 5-minute exposure in hot water at 47° C would kill the hyphae and conidia and a 10-minute exposure in hot water at 50° would kill the microsclerotia of V. albo-atrum. Similar results were obtained by the author using soaked cotton stem portions. Therefore it appeared that the temperatures developed by the four compost piles killed any living portion of V. albo-atrum. The temperatures developed by these semi-aerobic piles were low when compared with an anaerobic heap examined at one of the cotton gins in Mesilla Valley, New Mexico. Temperatures of 79.4° at the 6-inch depth were common and still higher temperatures were recorded below the 6-inch depth. In addition to eliminating the

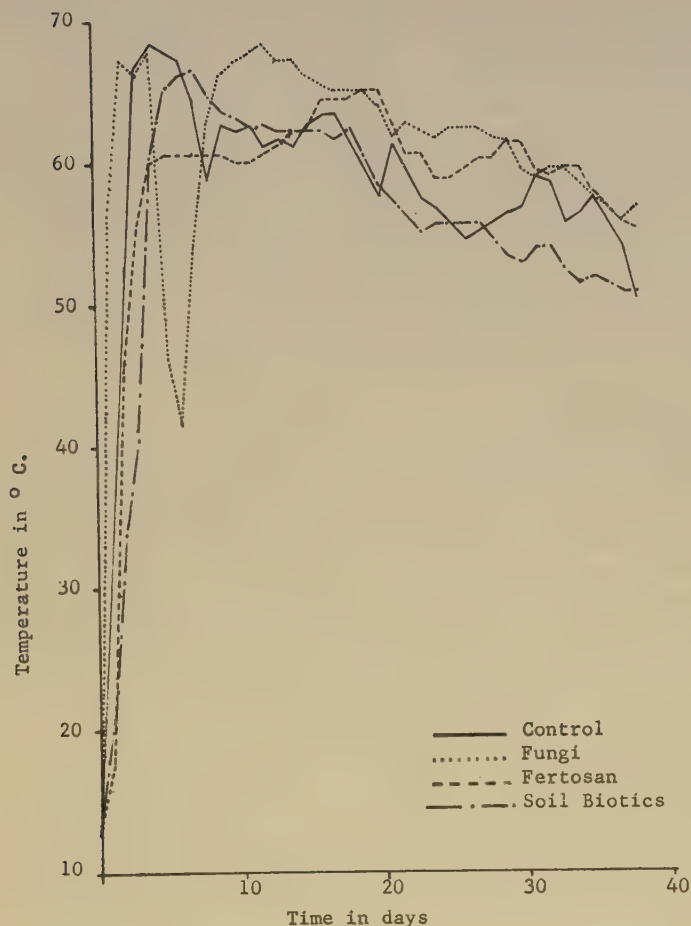


FIGURE 2. Temperatures developed in the three amended and the non-amended compost piles over a 38-day period.

Verticillium wilt organism, much of the morning glory (*Ipomoea* spp.) seed found in this gin waste was killed.

Composting of cotton plant residues following the ginning operations destroys the Verticillium wilt organism and eliminates it as a possible source of inoculum. The non-treated compost pile was as effective as the treated piles and would not present any problems in special handling of material during decomposition. The continued addition of non-treated, infested gin trash to productive land could lead to greater spread of wilt in New Mexico. Composting of gin trash prior to addition to soil could aid in reducing the problem of Verticillium wilt.

#### Acknowledgments

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STRAINS OF XANTHOMONAS VESICATORIA (DOIDGE)  
DOWSON DIFFERING IN VIRULENCE ON TOMATO AND PEPPER<sup>1</sup>

S. P. Doolittle<sup>2</sup> and D. F. Crossan<sup>3</sup>

Summary

Isolates of *Xanthomonas vesicatoria* from lesions on severely infected tomato fruits produced many typical bacterial spot lesions on tomato leaves, but only a trace of spotting on pepper foliage. Conversely, an isolate from pepper caused abundant spotting of pepper leaves but only a few lesions on those of tomato plants. This host specificity apparently is not characteristic of all isolations from tomato or pepper.

In July 1958 the senior author received tomato fruits showing many typical lesions of bacterial spot, caused by *Xanthomonas vesicatoria* (Doidge) Dowson. These fruits came from a field on the Eastern Shore of Virginia in which the crop had been seriously damaged by bacterial spot. Cultures made from fruit lesions produced typical colonies of *X. vesicatoria*. Four cultures from such colonies all caused typical symptoms of bacterial spot on leaves of Rutgers tomato plants inoculated in the greenhouse.

In November 1958 the four isolates were tested again in the greenhouse on sets of five Rutgers tomato plants (5 or 6 leaves) and two plants of California Wonder pepper (12 to 14 leaves). The inoculum was prepared by suspending the bacterial growth from three 48-hour-old agar tube cultures in 200 ml of sterile water; carborundum powder was added to the suspension to produce a slightly abrasive effect. The inoculum was sprayed on the plants with an atomizer at a pressure of 8 to 10 p.s.i. After inoculation the plants were held in a fog chamber for 48 hours and then returned to the greenhouse, where the temperature ranged from 23° to 28° C. This method of inoculation was used in all the later tests.

The inoculated tomato plants all showed abundant lesions on the leaves but one isolate, No. 6-1 from tomato, seemed somewhat more virulent than the other three. The pepper plants, however, showed only a trace of infection regardless of the isolate used. In this experiment the slight infection on pepper was attributed to the age and retarded growth of the plants rather than to any specificity of the isolations.

Shortly after the inoculations described were made, the junior author was asked for a virulent culture of *X. vesicatoria* and supplied one obtained from pepper in 1958. This culture, No. 58-1-r, and tomato culture No. 6-1 were tested in parallel inoculations on sets of five Rutgers tomato (5 or 6 leaves) and five California Wonder pepper plants (7 or 8 leaves). In this experiment the culture from tomato again produced many lesions on tomato leaves, but only a very few lesions on each of the pepper plants. In contrast, the culture from the pepper caused severe spotting of pepper foliage but only a trace of infection on tomatoes. The two cultures showed the same difference in later trials at Beltsville and in two tests made by the junior author in the greenhouse at the Delaware Agricultural Experiment Station.

In all experiments the authors evaluated infection on a scale of 0 to 5 in which 5 equalled severe leaf infection. Between November 1958 and June 1959, 34 tomato and 35 pepper plants were inoculated with culture No. 6-1 and 34 tomato and 34 pepper plants with culture No. 58-1-r. On tomato the average infection rating was 3.2 for the tomato isolate and 0.75 for the pepper isolate. On pepper the ratings were 0.5 for the tomato culture and 2.7 for that from pepper. The degree of infection on individual plants showed no extreme variation and the results were alike in all tests at both locations.

These results indicate that strains of *X. vesicatoria* differ in their ability to infect tomato and pepper. This difference, however, is not characteristic of all isolations of the organism. The senior author in the past obtained cultures of *X. vesicatoria* from both pepper and tomato that produced approximately equal amounts of infection on both hosts in the greenhouse. Gardner and Kendrick<sup>4</sup> also found that cultures from tomato and pepper both produced abundant infection on either of these hosts.

The strains used in the present experiments were not tested on a large number of differential media, but their appearance and rate of growth seemed to be identical in nutrient broth and on beef-infusion, Thaxter, and tomato-juice agars. On these media their characteristics were typical of those described for the type culture of *X. vesicatoria*.

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<sup>1</sup> Published with the approval of the Director of the Delaware Agricultural Experiment Station.  
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<sup>4</sup> Gardner, Max W. and James B. Kendrick. 1923. Bacterial spot of tomato and pepper. Phy-

EPIDEMIOLOGY OF STEM RUST OF WHEAT :  
III. MEASUREMENTS OF INCREASE AND SPREAD

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Summary

Intensification of stem rust was exponential within discrete quarter-acre plots. The rate of intensification within these plots and the rate of spread from plot to plot was influenced by wind direction and other prevailing meteorological conditions.

INTRODUCTION

The progress of an artificially induced epiphytotic of stem rust of wheat (*Puccinia graminis tritici* Eriks. & E. Henn.) was intensively studied at Fort Detrick, Frederick, Maryland in 1957 in quarter-acre plots of wheat arranged in a polar coordinate grid. The central plot was inoculated at tillering. Rust development was charted through the crop season in the central plot and in the other plots situated about it along radii following the eight principal points of the compass.

The experiment essentially parallels research conducted at St. Croix in the U. S. Virgin Islands from 1955 to 1957 in which spread and intensification of rust were measured in discrete fields across the Caribbean Island (4). Randomness of winds in Maryland dictated the use of a circular pattern of fields, whereas the nearly unidirectional trade winds of the Caribbean permitted orientation of fields in a line or arc downwind from the inoculated site. A much weaker spore source plot (1/4 acre instead of 6 acres for St. Croix) was employed in Maryland and plots were located closer to the source than at St. Croix.

This study of epiphytotic disease development formed a part of an integrated program of field experimentation with stem rust conducted at Fort Detrick during 1957. Data from three other separate but coordinated studies were employed in analyzing and reconstructing the progress of rust spread and intensification as it was influenced by windflow, temperature, and dew for initiation of new infection. One such concurrent study was the detailed examination of uredospore dissemination patterns arising from the central plot during the period in which only primary infection was present. This is reported by Bromfield et al. (1). In another study the relative favorability of nights for initiation of infection was investigated through microscopic examination of the leaves of uniformly inoculated seedling plants exposed each night to prevailing meteorological conditions. The third separate but coordinated study consisted of a series of "spore sandwich" inoculations during the season using the technique suggested by Geis et al. (2). By this technique, filter paper squares dipped in a spore suspension are appressed to the leaf surface with cellophane tape. It provided data on the relative length of the incubation period of the organism during portions of the crop season. Data from these studies have proved very useful in analyzing the progress of the epiphytotic and illustrate the fruitfulness of integrated experimentation of this type.

Hitherto studies of the epiphytology of wheat stem rust at Fort Detrick, Frederick, Maryland had been concerned solely with disease development within continuous stands of grain. Two major advantages accruing from use of scattered plots are a) the practicality of study of above-crop spore movement and b) virtual elimination of interplot contamination by individuals recording data.

MATERIALS AND METHODS

Nineteen plots, each of approximately 10,000 square feet were established on the Fort Detrick reservation in a polar coordinate grid consisting of two rings of plots at approximately 900 and 1800 feet from the central plot. Plots were aligned along radii corresponding to the eight principal points of the compass. An extra plot was added in the southeast quadrant. Space permitted inclusion of an additional plot nearly 2700 feet southwestward of the focus.

All plots were sown to Knox (CI 12798) winter wheat in mid October 1956 at 6 pecks per acre in rows 7 inches apart. Knox was selected because of susceptibility to race 56 of stem

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rust combined with resistance to naturally occurring races of leaf rust and powdery mildew.

Details on the method of inoculating the central plot, hereinafter referred to as the "focus," are given by Bromfield et al. (1).

An AN/GMQ-1 anemometer<sup>5</sup> (5-foot height) was operated at a location 200 feet from the focus plot during the test period. Rainfall was measured by a standard rainfall gauge. Hygrothermographs were operated in one plot to record temperature and relative humidity in the zone of plant growth. A Taylor dew meter in the plant stand characterized dew deposition and the time of occurrence of rain showers (5).

Observations for the presence and amount of rust infection were made at 2- to 3-day intervals. Readings were made at ten locations arranged in an "X" pattern in each plot. Units of 20 culms per sampling point were examined until such a time as numbers of pustules rendered counting impractical. Subsequently, estimates of severity were made. Ten pustules per culm were considered equivalent to 1 percent severity in relating the two measures of disease in the graphs of disease intensification presented in this paper. Instances in which independent pustule count and severity estimates of the same plant material were obtained bear out the validity of this assumption (3).

Extreme care was taken by persons recording data to avoid interplot contamination. Personnel did not enter plots considered free of rust except when clothed in freshly laundered coveralls, caps, and cloth boots. The cloth boots covered the person's shoes and enclosed the leg ends of overalls. Complete changes of clothing were made before entering individual plots, even if no rust was encountered, except when appreciable amounts of rust were known to exist within a group of plots.

## RESULTS

### The Focus Plot

"Flecking" was first noted in the central or "focus" plot on May 2, nine days after inoculation. One or 2 days later pustules had ruptured plant tissues and begun to shed uredospores. Plants were inoculated when 8 to 10 inches tall and in the tillering stage; hence, the primary infection was concentrated on the four lower leaves of the plants. The oldest of these leaves had begun to turn yellow and wither during the first week in May, due both to shading and to the heavy disease infection. The level of primary infection in the focus as determined on May 7, 10, and 15 averaged 11 to 13 pustules per culm (approximately 1 percent severity according to the conversion ratio).

Secondary infection became evident on May 17 with the appearance of new flecks and pustules. Plants were then 32 to 42 inches tall and just past flowering. Much of this new cycle of disease appeared on stem tissues and some higher leaf blades. Intensification in the focus was rapid; rust severities for May 15, 23, 28, 31, June 6 and 12 were 1, 15, 30, 40, 60 and 80 percent respectively. Plants were prematurely killed by rust in the focus 4 days to a week before death occurred to wheat in surrounding plots. Plots in which rust did not develop matured normally.

The pustules that became evident on May 17 apparently originated from infections on the nights of May 8 and 9. Sufficient moisture and early morning temperature and light for the establishment of some infection occurred on both of these nights. Conditions from May 2 through May 6 were not favorable for infection. Meteorological conditions were favorable for some infection on May 8 and 9 and somewhat less favorable on May 7. Infection in plots at some distance from the focus was also located which corresponded with wind directions on these two dates.

The increase of infection in the central plot is presented graphically in Figure 1. The ordinate extends from 0.01 pustule per culm (p/c) through 100 percent severity (1000 p/c = 100 percent). Data from observations during the incubation period of primary infection have been displaced to the right in time to a date prior to appearance of secondary infection, since no pustule increase can occur during the initial incubation period.

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<sup>5</sup> This consists of a mast and wind transmitter as supplied by the Army Signal Corps modified by a specially constructed bridge and rectifier controlling two Esterline Angus recording milliammeters.

### Rust in Outlying Plots

The movement of spores from the area of initial infection was first in an arc east, north-east and southeast of the focus. Later spread occurred into plots lying in an arc encompassing fields lying southwest through south. It was not until June 10, close to maturity of the crop, that rust finally was found in the plots lying west and northwest of the focus. Prior to discussion of these movements, wind characteristics of the test period will be presented.

Distribution of daytime winds (0700-1900) passing over the plots for the period May 2 through June 2, 1957 for those portions of the period when the plants were free of surface moisture is presented in Table 1. Wind directions for days when the humidity was near 100 percent during most of the day were not included in the table since no appreciable spore dissemination occurred under those conditions. Negligible numbers of spores were collected on rainy days (1). Dews usually disappeared between 0700 and 0800 EST. No correction was made for these short periods of moisture since air movement was less pronounced at this time than it was later in the morning.

Table 1. Hourly distribution of daytime winds during the period that leaves were dry.

Date	: Calm : (2 mph or less)	Wind direction (+ 22 1/2°)							
		N	NE	E	SE	S	SW	W	NW
May 2	3	0	3	2	1	0	0	0	0
3	2	3	4	3	0	0	0	0	0
4	1	3	4	4	0	0	0	0	0
5	7	1	3	1					
6	2			1				4	5
7						1	3	7	1
8	1					4	5	2	
9	1					4	4	3	
10	1					6	6		
11 & 12 rain									
13					1		5	6	
14 rain									
15							10	2	
16		1	7	2			1		
17		3	1		3	5			
18	1		6	2	2	1			
19-22 rain, high humidity and low temperatures									
23						9	3		
24								4	8
25		1	2	2	2	2	3	2	
26						7	5		
27	1					1	4	5	1
28		1						1	10
29	1		1	6	1	3			
30	2					6	4		
31			5		2	4	1		
June 1	2				8	2			
2		2				3	2	2	3
Entire period <sup>a</sup>	8%	5%	12%	8%	7%	20%	19%	12%	9%

<sup>a</sup>Percentage of total winds by directions plus calm.

Certain dates, such as May 18 and 25, were characterized by extremely variable windflow. South, northeast, southwest and west winds were most prevalent during the test interval in the order named, yet each direction was represented at least 5 percent of the time.

Rust was initially discovered in the five plots located NE, E and SE of the focus on May 22. The pustules found on May 22 were well developed and of the same generation or generations responsible for the initial secondary break in the focus plot. Mean levels of infection of rust in the more distant plots (1800 feet) were lower than those obtained in the same direction but 900 feet from the spore source.

Evidence that the NE, E, SE arc of spread actually had occurred to a lesser extent in the

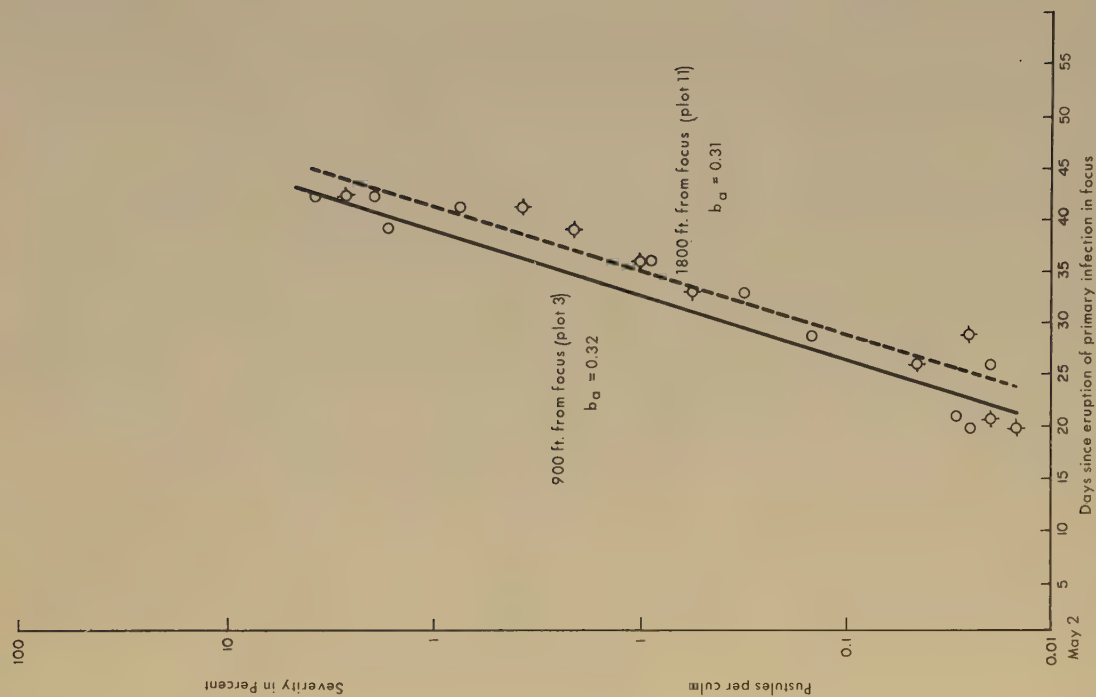


Figure 2. RATE OF RUST INTENSIFICATION NORTHEASTWARD FROM FOCUS

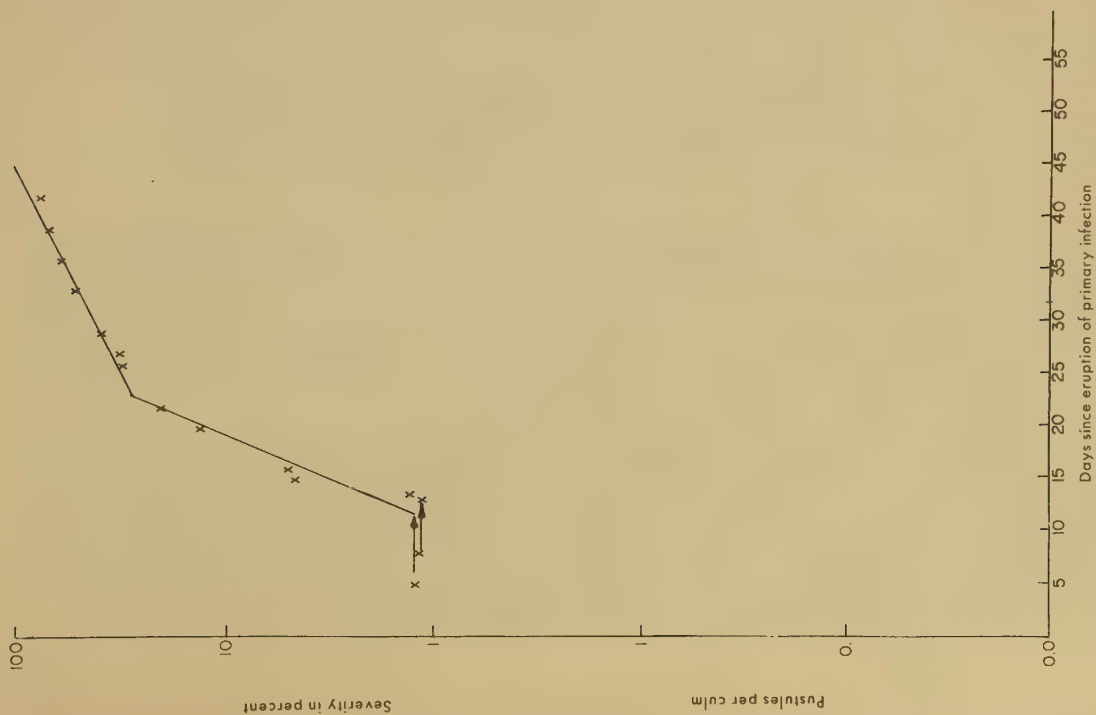


Figure 1. RATE OF RUST INTENSIFICATION IN THE FOCUS

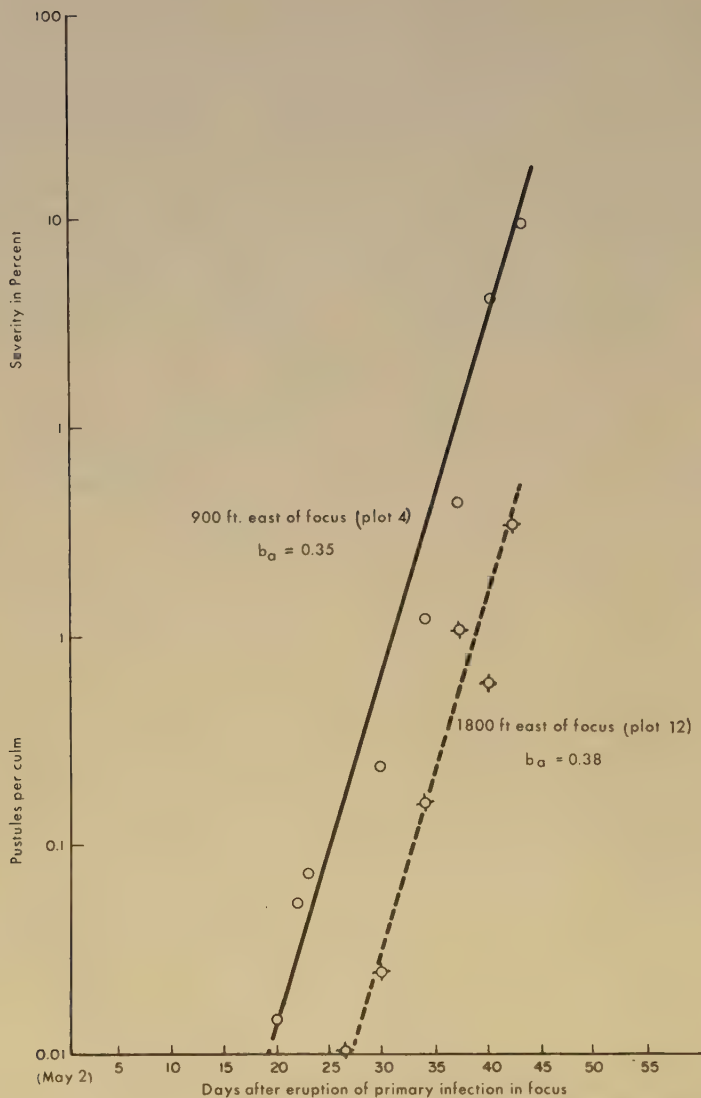


Figure 3. RATE OF RUST INTENSIFICATION EASTWARD FROM FOCUS

sector northwards was obtained on May 28, 29 and 31. An older pustule was found May 28 in the plot 1800 feet north. The following day another well developed pustule was found in the plot 900 feet north. On May 31 a cluster of four pustules, one old and three very young, were found at 900 feet, suggesting that infection had occurred in the two northern plots sufficiently long to reproduce there, probably May 8 or 9. On May 31 a new outbreak of infection was discovered in the plots NE, E and SE. Thus, it appears that initial development of infection N through E and SE occurred on the same day or days.

Wind distribution records indicate that spores causing the N-SE arc of spread could have been dispersed each day from May 6 through May 10. Detailed spore trapping about the focus during this interval (1) showed acceptable trajectories, but negligible spore movement on May 6 and 7. Examination of meteorological records indicated that no infection occurred on May 6 or 10 because of inadequate dew. A slight amount of infection may have occurred on May 7, with somewhat better infection on May 8 and 9. It appears therefore that disease development in the N through SE arc was the result of weak infection outbreaks initiated on May 8 and 9, and perhaps on May 7 also.

The second distinct disease development occurred in plots lying SW and S of the focus. This area of infection was first indicated by detection of some young flecks on May 28, in the SW plot only 900 feet from the focus. By the following day (May 29) pustules were further developed and easier to discern. Pustules were found as far as 2700 feet from the focus along the SW line of three plots and lesser amounts were detected in some of the plots southward from the focus. Mean infection levels for May 29 and 31 indicated a decrease in intensity with increase in distance from the source of spores along the southwest line of fields:

<u>Distance from focus plot, feet</u>	<u>Mean infection, pustules/culm</u>
900	0.053
1800	0.020
2700	0.009

The spores dispersed on May 16 from the focus into the SW and S plots initiated the infections in this area. Wind records show that spores could also have been carried SW and S on May 17 and 18, but both nights were unfavorable for infection.

Rust did not appear in the four W-NW plots until June 10, only a few days before yellowing of plants preceding normal ripening. This, at first, seems rather surprising since about 15 percent of the total daily winds could have carried inoculum from the source to these plots. Closer scrutiny of infection potential on nights following days of such windflow indicates that absence of rust would be expected. Meteorological conditions were not favorable for infection on nights when uredospores were deposited in the area during the periods of May 2-6 and 17-18. Inoculum that eventually caused the pustule break noted in June could have been produced in plots other than the central one. The many instances of negative counts would seem to remove doubt that infection had existed earlier but in such low intensities as to escape detection in the four plots.

Figures 2 and 3 are graphs of rust development in fields located northeastward and eastward of the focus plot, respectively. They are typical of other graphs that could be presented for development in plots in other directions.

Terminal severities of from 5 to 12 percent were obtained in the plots of the inner ring that received infection from the two periods of spread.

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EPIDEMIOLOGY OF STEM RUST OF WHEAT:  
IV. THE USE OF RODS AS SPORE COLLECTING DEVICES IN A STUDY ON THE  
DISSEMINATION OF STEM RUST OF WHEAT UREDOSPORES

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Abstract

Adhesive-coated rods (1.6 mm in diameter), silicone-greased microscope slides, and wheat seedlings were used to trap stem rust of wheat uredospores in the vicinity of a plot of rusted wheat. The numbers of spores collected on coated vertical rods were more closely correlated with the numbers of resulting pustules than were spore numbers collected on greased slides positioned horizontally.

The greatest number of uredospores was generally trapped during the period 1100 to 1500 hours EST. Usually the plant surfaces in the source area during this period were driest, winds tended to be highest, and relative humidities lowest.

INTRODUCTION

A simple and reliable device to indicate the number of airborne spores being deposited on plant surfaces is needed to facilitate epidemiological studies of many plant diseases.

Gregory (1) has shown that small diameter cylinders (0.018 to 2.0 cm) covered with adhesive material are efficient spore-trapping devices. In general, the collecting efficiency of such cylinders increases with increasing wind speeds up to at least 20 miles per hour.

Since spore deposition on plant surfaces varies with wind speed it was thought that a valid correlation might exist between numbers of spores trapped on vertical cylinders and numbers of spores deposited on wheat plant surfaces under field conditions. If such a relationship were found, then spore counts obtained from cylinders could be used to predict the number of pustules that could be expected per leaf of wheat plant if environmental conditions influencing germination, penetration, and establishment were known (3). A preliminary investigation of small coated rods for this purpose was begun at Fort Detrick in the spring of 1957 as one phase of an integrated study concerning the spread of stem rust of wheat from an inoculated plot.

MATERIALS AND METHODS

Source Plot -- Description and Treatment

A rectangular plot 80 by 90 feet was planted to Knox wheat (C. I. 12798) in the fall of 1956. Hereafter this plot will be referred to as the "source plot" or "focus." On the evening of 23 April 1957 the wheat, then in the tillering stage, was inoculated with stem rust of wheat race 56. Uredospores, thoroughly mixed with talc, were dusted with a rotary hand duster onto the plants covered with polyethylene plastic sheets (40 x 100 feet). The plastic sheets were weighted down by sections of cement reinforcing rods. The plastic was left over the plants until 0800 the following morning to provide suitable moisture and temperature conditions for spore germination and subsequent rust penetration. By 0800 temperature under the sheets had risen to, but had not exceeded, 85° F. On May 2, 9 days after inoculation, pustules were first observed in the inoculated plot. On May 10 the average rust severity in the focus was 1 per cent.

Spore Sampling Stations -- Arrangement and Description

Eight sampling stations (#1 to #8) were established in a ring around the source plot described above. Each station was located 150 feet from the center of the source plot. An additional station, #9, was located in the center of the source plot. The bearing of each station

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<sup>5</sup>Quality Maintenance and Improvement Section, Agricultural Marketing Service, United States Department of Agriculture, Beltsville, Maryland.

(true north being 0°) is given as follows:

Sampling station number	Bearing of station from center of source plot
1	11°
2	41
3	93
4	144
5	189
6	218
7	293
8	346
9	Center of source plot

Stations 1 through 8 were located on the bearings indicated in order to align them with outlying plots of wheat previously established at distances of 900 feet and 1800 feet, respectively, from the center of the source plot. Rust spread to and development in outlying plots around this source plot is discussed in a paper by Underwood et al. (4).

Figure 1 shows the slide, rod, and wheat seedlings used as spore-trapping devices and the method of exposure at stations 1 through 8. At station 9 the same devices were used but instead of being placed on the ground they were put on a 2-foot-high wooden support which raised them slightly above the tops of the wheat plants in the source plot.



FIGURE 1. Spore collecting devices used at the sampling stations.

#### Sampling Devices -- Description and Treatment

**Rods:** The cylinders used for air sampling were stainless steel rods 1.6 mm in diameter and 13 cm long. Each rod was inserted into a #5 cork stopper so that about 1.5 cm extended beyond the large end of the cork. Rods were coated with an adhesive by dipping the long end into a mixture composed of 2 parts gelatine, 7 parts glycerine, 6 parts water and 1 percent phenol kept liquid in a steam heated water bath. After coating, each rod was inserted into a test tube 15 cm long by 1.5 cm in diameter. This protected the coated rod from contamination before and after exposure.

The rods were exposed in the field by inserting the short uncoated portion into a small hole bored into a wooden platform which was held above ground level by an inverted 4-inch flower pot. The cork acted as a base to maintain the rod in a vertical position. After exposure the rods were reinserted into the protective test tube and taken into the laboratory for assessment.

Direct counts obtained by microscopic observation of spores collected on the rods were made but proved inadequate. Higher counts were obtained from these rod areas when the coating (and spores) was carefully removed, dissolved in water on microscope slides, and the spores observed and counted. The latter procedure was slow, tedious, and unsuitable for routine use.

Additional methods for removing adhesive and spores or spores only from the rods were tried. The method finally adopted included the use of a roll of 2-cm wide Scotch tape mounted |

in a large office-type dispenser. A section of tape was stretched from the roll to the cutting edge and fastened. This gave a section of tape about 3 inches long with the adhesive surface down. The rod to be examined was removed from its protective tube and placed beneath the tape in such a manner that its long axis was at right angles to the long axis of the strip of tape. The rod was raised, placed against the adhesive surface of the tape, and rolled in one direction for a minimum of four revolutions. In effect, the spores and other particles were removed from the surface of a cylinder 2 cm in height and 1.6 mm in diameter. Such a cylinder has a surface area of approximately 100 mm<sup>2</sup>.

The portion of the tape which had been in contact with the rod was then cut from the roll and mounted adhesive side down in a large drop of lacto-phenol and cotton blue on a microscope slide.

The entire area under the tape was examined under the low power of the microscope (10X eyepiece) and all rust spores counted and recorded. Examinations were made of areas of the rods from which spores had been removed by the Scotch tape adhesive. In all cases practically all particles had been removed by the adhesive of the tape. Only an occasional spore was ever found on rod areas so treated.

Duplicate mounts were made from 32 rods. One mount was made from an area slightly above, the other slightly below, the mid-point of the exposed rod. Statistical analysis of the counts indicated no significant difference existed between paired counts. In view of close agreement between duplicate mounts, each rod count recorded in this paper is based on the results obtained from a single mount, that is, a cylinder 2 cm high (100 mm<sup>2</sup> surface area) near the midpoint of the rod.

Slides: The glass slides exposed at the sampling stations were conventional microscope slides 3 inches by 1 inch by 1/16 inch. All slides were coated with a thin, even film of Dow Corning Silicone Stopcock grease. Slides were numbered and greased in advance of use and stored in wooden microscope slide boxes equipped with tight fitting lids. Groups of nine slides were placed in individual boxes for convenience in placing and retrieving them in the field.

Slides were exposed horizontally on a wooden platform (4 1/2 inches square) bolted to the base of an inverted 4-inch clay pot (Figure 1). The long axis of the slide was in line with the radius extending from the center of the source plot.

Counts of uredospores trapped on the slides were made with microscopes equipped with 10X oculars and 10X objectives. Five swaths, each 40 mm long by 1.5 mm wide, were observed on each slide. Thus a total area of 300 mm<sup>2</sup> was scanned per slide.

Seedlings: Seven- to 10-day-old seedlings of the variety Baart (C.I. 1697) were used. Fifteen seeds per 4-inch clay pot were planted in a line and the number of plants trimmed to 10 per pot prior to exposure. All secondary leaves were also removed. Before field exposure plants were grown in a rust-free greenhouse whose incoming air was filtered to prevent possible rust contamination. Plants were carried to the sampling stations in the field in a covered box to reduce contamination. At sampling stations, pots were placed with the line of seedlings at right angles to the radius extending from the center of the source plot.

After field exposure the pots were collected, placed in a box and returned to the greenhouse. All exposed plants from the morning and afternoon sampling periods discussed below remained on the greenhouse bench until the plants exposed from 1500 to 1900 hours were retrieved.

At 1900 the exposed plants were placed in a dew chamber controlled at 72° F for a 14-hour dew period. These conditions provided near optimum conditions for spore germination and penetration.

After the requisite dew period the plants were placed in a greenhouse for pustule development to occur. Counts of the numbers of pustules on each leaf were made 10 days later and the results recorded.

#### Sampling Periods and Sampling Time

Sampling was begun on May 6. By this time most of the pustules resulting from the initial infection had ruptured the host plant epidermis and were shedding spores. Sampling was continued daily until May 14. During this period only pustules of the initial or primary cycle were producing spores. Therefore variations in numbers of spores trapped at the central station (plot focus) were primarily a reflection of environmental conditions rather than a result of increase in numbers of pustules.

Rods, slides, and seedlings were exposed for three 4-hour periods during each day (0700-1100, 1100-1500, 1500-1900 hours EST). Only rods and slides were exposed during the night from 1900 to 0700.

### Weather during Sampling Period

At the start of the sampling period on May 6, 1957 the entire eastern half of the United States was under the influence of a large high pressure system centered in Kansas and Missouri. The relatively dry continental polar air mass associated with the high persisted at Frederick throughout the period May 6 to May 10. Each of these days was meteorologically quite similar. During this period the center of the system moved eastward through Tennessee and South Carolina and by May 10 was well off the coast of the Carolinas. The pressure gradients during this time were rather weak and the winds at Frederick were predominantly from the quadrant south through west. A trace of rain was recorded in a small shower occurring at 1700 hours EST on May 6. This was the only precipitation during this period. Nights were clear and dews were observed on the nights of May 7, 8, 9, and 10.

The weather during daylight hours in this period was similar for each of the 5 days. Skies were practically clear on May 6, 7 and 8. Only a few small cumulus clouds formed in the afternoon on these days and the sky coverage never exceeded 1/10. A greater development of cumulus was observed on the 9th (maximum 4/10 sky coverage) and on the afternoon of the 10th cumulus clouds plus a layer of higher clouds combined to give a maximum sky coverage of 8/10. Winds were generally calm in the early morning, rising to a maximum of about 10 mph in the afternoon with occasional gusts up to a maximum of about 20 mph. With the approach of evening, winds again subsided to calm.

There was a gradual warming trend from May 6 to May 10 (May 6: min. 50° F, max. 73° F; May 10: min. 58° F, max. 90° F).

During Saturday, May 11, a cold front moved through Frederick from the north and winds at this time shifted from the south to the north and northeast. Relative humidity remained high throughout the day and water was present on the plants in the source plot except for a period of about 4 hours from 0900 to 1300 hours EST. The cold front became stationary in the vicinity of Frederick during May 12. Fog and drizzle occurred during the morning hours. In the afternoon the cloud cover dissipated somewhat, temperature rose, and the relative humidity dropped. The wind direction also shifted from north to southeast although the wind speed was only 5 mph or less.

During the early morning hours of May 13 the front moved somewhat north of Frederick. Clouds were scattered (2-5/10 coverage), temperature rose to about 90° F in the afternoon and the relative humidity dropped to about 50 percent. The late morning and afternoon winds, generally from the south and southwest, were gusty and averaged from about 5 to 10 mph. A light rain occurred at about 1500 hours.

During the evening of the 13th the front again moved south through Frederick and by 0130, May 14, it was aligned east-west through Washington, D. C. where it remained as a stationary front for the rest of the day. During the 14th the winds were from the north to northeast, gusty, and averaging 3 to 10 mph. Rain fell at 0500-0600, 0800-0900 and again at 1700-1900 hours EST. The relative humidity remained at 100 percent during the day except for the period 1100-1700 hours when it dropped to about 80 percent as the cloud cover broke and temperature rose to a maximum of 80° F at 1400 hours.

On each of the nights during the sampling period, with the exception of the night May 6-7, dew or rain traces were recorded on a Taylor dew meter for most of the hours of darkness. In general, the winds were calm during the hours of darkness.

### RESULTS

The design of this experiment necessitated the elimination of all spore-trapping data not related to the spore-transporting wind direction. For the statistical analysis only values obtained from sampling stations with an arc plus or minus 2 standard deviations from the daily mean wind direction were considered<sup>6</sup>. This procedure eliminated the obligate zero values introduced into the experiment by the sampling pattern.

Table 1 shows the number of pustules per leaf obtained as a result of exposure at each of the sampling stations during the 9-day sampling period. Values for sampling stations within the arc plus or minus 2 standard deviations from the daily mean wind direction are underscored. From these data it can be seen that seedling leaves positioned outside this arc (notably at stations 5, 6, 7 and 8) trapped relatively few spores, as is indicated by the small numbers of

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<sup>6</sup>On May 9 and 10th the wind directions were bimodal and values within plus or minus 2 standard deviations from both means were considered.

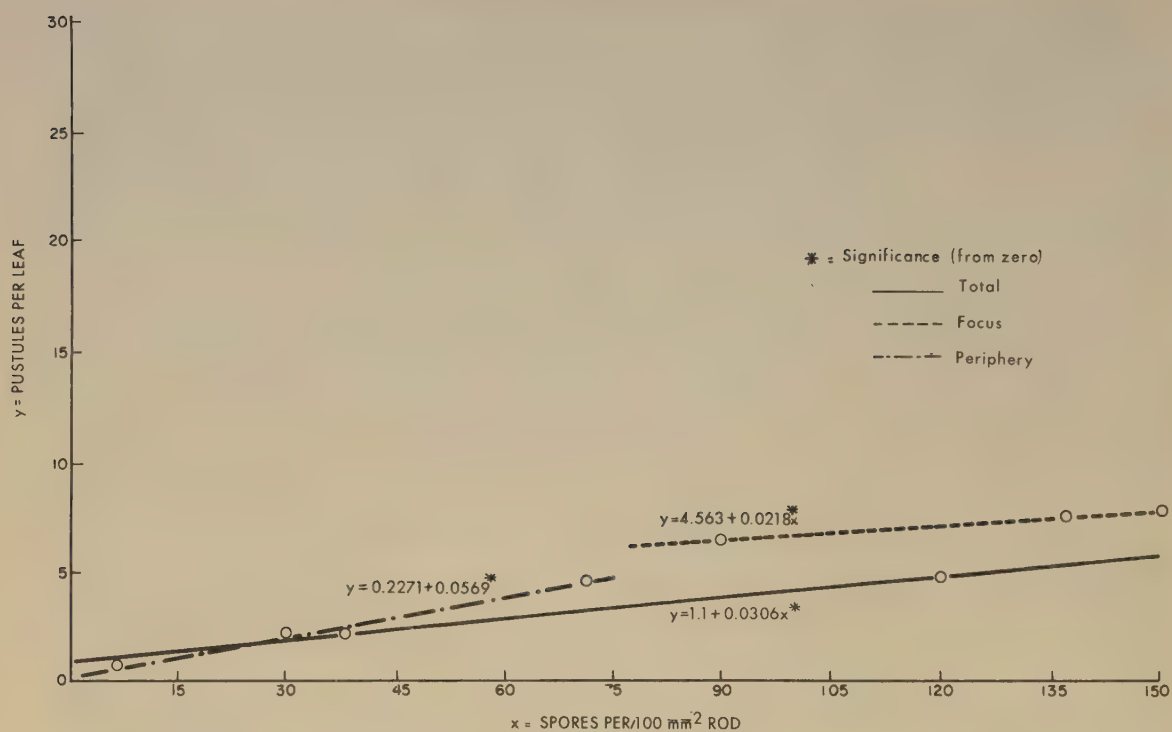


Figure 2. REGRESSION COEFFICIENT OF SPORES/100 mm<sup>2</sup> ROD VERSUS PUSTULES PER LEAF

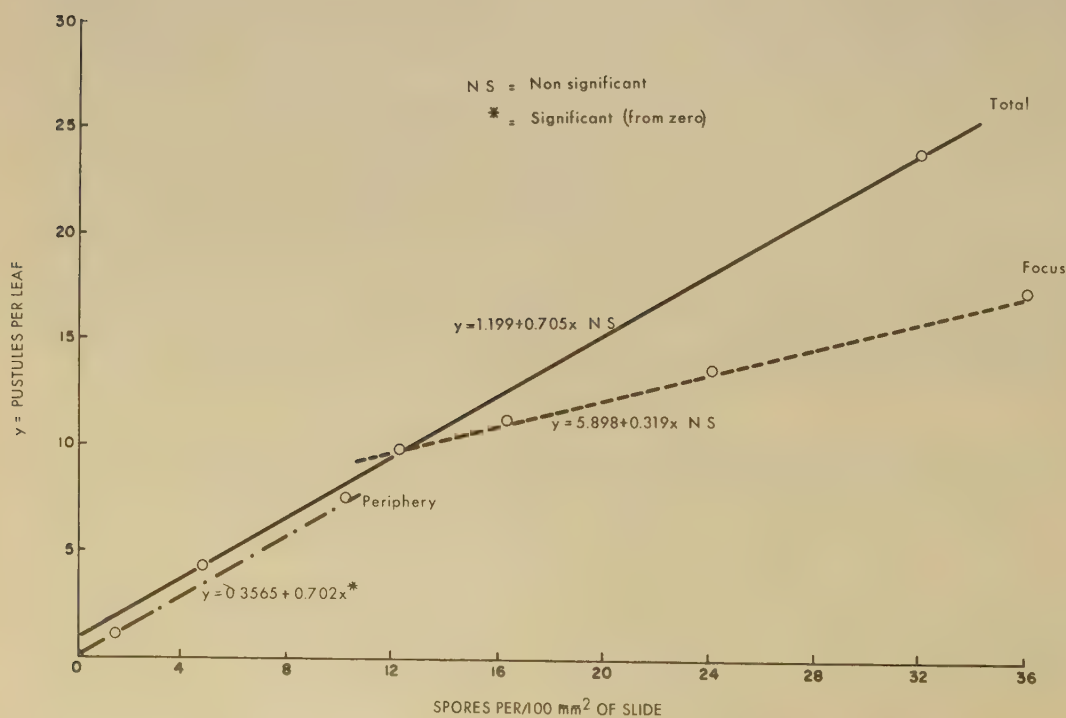


Figure 3. THE REGRESSION COEFFICIENT OF SPORES/100 mm<sup>2</sup> OF SLIDE VERSUS PUSTULES PER LEAF

Table 1. Number of pustules per leaf obtained at each sampling station during the 9-day sampling period.

Date	Sampling station								
	#9 (Focus) <sup>a</sup>	#1 11°	#2 41°	#3 93°	#4 144°	#5 189°	#6 218°	#7 293°	#8 346°
May 6	5.3 <sup>b</sup>	0.1	0.2	<u>0.1</u>	<u>0.5</u> <sup>c</sup>	0.0	0.1	0.1	0.5
7	8.0	0.0	<u>0.5</u>	<u>1.2</u>	<u>0.3</u>	0.0	0.0	0.0	0.1
8	34.3	<u>2.3</u>	<u>6.1</u>	<u>2.5</u>	<u>0.3</u>	0.0	0.0	0.1	0.0
9	47.4	<u>2.0</u>	<u>7.9</u>	<u>2.6</u>	<u>0.4</u>	0.2	0.1	0.2	<u>0.4</u>
10	33.6	<u>2.6</u>	<u>6.3</u>	<u>2.3</u>	<u>0.4</u>	0.2	0.2	0.4	<u>0.2</u>
11	6.4	<u>0.1</u>	<u>0.0</u>	<u>0.2</u>	0.0	<u>0.9</u>	<u>0.7</u>	0.1	0.2
12	14.6	<u>0.9</u>	<u>0.5</u>	0.0	0.0	<u>0.0</u>	<u>0.0</u>	0.1	<u>0.8</u>
13	37.3	<u>0.4</u>	<u>0.4</u>	<u>4.5</u>	<u>1.3</u>	0.0	0.1	0.0	<u>0.2</u>
14	2.8	<u>0.3</u>	<u>0.0</u>	<u>0.0</u>	<u>0.1</u>	<u>0.2</u>	<u>1.2</u>	0.0	0.0
Total		8.7	21.9	13.4	3.3	1.5	2.4	1.0	2.4

<sup>a</sup>Inoculated central plot.<sup>b</sup>Pustules per leaf.<sup>c</sup>Values underlined are those within the arc  $\pm 2$  standard deviations of the mean wind direction.

pustules. Actually 92.9 percent of the pustule population occurred on the wheat leaves located within the plus or minus 2 standard deviations arc.

The number of spores trapped by rods, slides, and leaves at sampling stations within the arc plus or minus 2 standard deviations from the daily mean wind direction are presented in Table 2. From these data the following statistics have been calculated:

For Station 9 in the Center of the Focus: The mean number of spores per 100 mm<sup>2</sup> of rod was 138 and the regression coefficient of spores per rod versus pustules per leaf is significant ( $b = 0.0218$ ). The mean number of spores per 100 mm<sup>2</sup> of slide was 16 and the regression of spores per slide versus pustules per leaf was nonsignificant ( $b = 0.319$ ). From these two regressions it is indicated that rods give a more reliable estimate of resulting pustules per leaf than do glass slides, when relatively concentrated spore clouds are involved.

For the Peripheral Stations: The regression coefficient of spores per 100 mm<sup>2</sup> rod versus pustules per leaf is significant,  $b = 0.0569$  as is the number of spores per 100 mm<sup>2</sup> slide versus pustules per leaf ( $b = 0.702$ ). (Table 3 and Figs. 2, 3). Hence, both sampling devices are adequate for estimating the resulting number of pustules per leaf when sampling clouds with spore concentrations similar to those encountered in this phase of the experiment, that is, 5 spores per 100 mm<sup>2</sup> slide and 37 spores per 100 mm<sup>2</sup> rod. The greater mean value of spores trapped per 100 mm<sup>2</sup> rod suggests, however, that rods would be more reliable than slides for sampling spore clouds less concentrated than those encountered here.

## DISCUSSION

In view of the results obtained in this single preliminary trial, it is concluded that coated stationary rods have potential value as spore traps in rust epidemiological studies. Their simplicity, low cost, and ease of use, and especially their efficiency, recommend them for employment in spore collecting studies where greased microscope slides are now used.

Numbers of spores trapped on rods correlated, in general, quite well with numbers of pustules resulting on seedling leaves exposed in groups of 10 in 4-inch clay pots. This indicates that further tests to investigate relationships that may exist between numbers of spores trapped on rods and numbers of spores deposited on wheat plants in field stands are warranted.

In this trial it was assumed that the number of pustules which developed on exposed leaves after a standard incubation treatment reflects the number of spores originally deposited on those leaves. Perhaps day to day differences in temperature, light, and relative humidity may so modify the infection potential of spores that this relationship is not sufficiently constant to permit a valid correlation between pustules per leaf and spores caught per rod. Investigations of the effect of environmental conditions on infection potential are needed to help clarify this point.

Table 2. Numbers of uredospores collected on 100 mm<sup>2</sup> areas of rods and slides and numbers of pustules per leaf obtained at sampling stations within arcs + 2 standard deviations from the daily mean wind directions.

Date	Sampling period	#9 (Focus)		#1			#2			#3			#4			#5			#6			#8		
		La	Sb	Rc	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R		
May 6	0700-1100	0.1	8	--							0.0	0	0	0.0	0	0								
	1100-1500	4.1	21	325							0.1	5.0	9	0.4	5	50								
	1500-1900	1.1	15	106							0.0	0	0	0.1	1	30								
7	0700-1100	1.2	6	36				0.0	0	0	0.0	0	2	0.1	0	7								
	1100-1500	6.1	18	--				0.0	3	4	1.2	5	24	0.2	1	0								
	1500-1900	0.7	1	13				0.5	1	0	0.0	1	0	0.0	0	0								
8	0700-1100	3.8	2	188				0.0	0	0	0.3	0	3	1.6	1	5								
	1100-1500	24.0	18	337				0.6	0	8	3.4	2	43	0.5	0	8								
	1500-1900	6.5	3	71				1.7	2	6	2.4	0	16	0.4	0	2								
9	0700-1100	11.9	42	247				0.0	0	0	0.0	0	6	0.4	0	28	0.1	0	33					
	1100-1500	16.4	14	869				0.4	2	3	2.7	5	23	1.6	5	32	0.1	0	2					
	1500-1900	19.1	10	130				1.6	3	11	5.2	1	23	0.6	0	1	0.2	0	0					
10	0700-1100	12.0	80	148				0.6	0	2	2.3	4	19	0.2	0	1								
	1100-1500	9.0	11	294				0.0	0	2	1.4	1	19	2.0	7	30								
	1500-1900	12.6	28	101				2.0	2	9	2.6	3	26	0.1	0	0								
11	0700-1100	1.9	4	9													0.0	0	0	0.3	0	0		
	1100-1500	3.6	28	6													0.8	0	0	0.0	2	0		
	1500-1900	0.9	6	3													0.1	4	0	0.4	0	0		
12	0700-1100	0.1	1	2				0.0	0	1	0.0	0	0											
	1100-1500	1.1	34	14				0.0	1	1	0.1	3	0											
	1500-1900	13.4	2	48				0.9	0	3	0.4	0	0											
13	0700-1100	17.9	10	217				0.4	0	3	0.3	0	2	2.4	0	12	0.0	0	0					
	1100-1500	18.5	29	153				0.0	2	0	0.0	0	0	2.1	4	25	1.0	0	9					
	1500-1900	0.9	--	--				0.0	--	--	0.1	--	--	0.0	--	--	0.0	--	--					
14	0700-1100	0.0	0	9													0.0	0	--	0.1	0	--		
	1100-1500	2.3	5	9													0.1	0	3	1.0	1	3		
	1500-1900	0.5	16	17													0.0	--	--	0.1	--	--		

<sup>a</sup>Figures shown in columns headed by "L" are average numbers of pustules per leaf. They are based on counts of 10 leaves at each station for each sampling period.

<sup>b</sup>Figures shown in columns headed by "S" are numbers of spores per 100 mm<sup>2</sup> of slide area. Actually the total number of spores in 300 mm<sup>2</sup> area of slide were counted and these values were converted to spores per 100 mm<sup>2</sup>.

<sup>c</sup>Figures shown in columns headed by "R" are total number of spores counted per 100 mm<sup>2</sup> of rod area, that is, on the surface of a cylinder 2 cm high and 1.6 mm in diameter.

Table 3. Regression coefficients.

Regression coefficients of	
Spores/100 mm <sup>2</sup> of slide versus pustules per leaf	Spores/100 mm <sup>2</sup> of rod versus pustules per leaf
Focus -- 0.319	0.0218 <sup>a</sup>
Periphery -0.702 <sup>a</sup>	0.0569 <sup>a</sup>
Total -- 0.705	0.0306 <sup>a</sup>

<sup>a</sup>Significant from zero.

An obvious advantage of rods over slides for the trapping of air-borne uredospores is the greater trapping efficiency of the former under field conditions. For example, in 32 instances during the 9-day trapping period at least one spore was trapped on a 2-cm length of rod when none was observed on 300 mm<sup>2</sup> area of a slide exposed concurrently. The reverse situation, spores observed on slide but not on rods, occurred 23 times. When maximum or average numbers of spores caught per unit area of slide are considered the greater efficiency of the rod is obvious.

The greater efficiency of the rods suggests that they would be useful devices to indicate the early appearance of spores in an area at concentrations lower than could be detected by the conventional slides now used.

The results of this test are in agreement with previously published reports which assign rust uredospores to the "day spora" group of Gregory (2). Although uredospores were occasionally trapped at night they were far fewer in number than those trapped during daylight intervals. In this trial the winds were mostly calm and dew or moisture in some form was present on the leaves during each of the nights that trapping was done. Thus the low numbers of spores in the air at night may be due to lack of sufficiently strong winds, high humidity, or liquid water, working alone or in combination. Another possible factor which must be considered is the presence or absence of releasable uredospores in the pustules during the night. Are mature uredospores present for distribution at night if weather conditions are favorable? The entire question of uredospore maturation cycles is an area requiring more intensive investigation.

In this test the greatest numbers of spores were trapped, in general, during the interval 1100-1500 hours EST. This was apparent at the center of the source plot and at the sampling stations 150 feet from the source-plot center. In some instances the next highest counts were obtained for the morning sampling interval (0700-1100) but in others they were obtained for the evening interval (1500-1900). Shorter sampling intervals, that is, 1 hour, appear to be necessary to show the diurnal trend of spore dissemination and to show how this trend is modified by environmental factors. At present it seems reasonable to conclude that greatest spore dissemination generally occurs at about noon when plant surfaces in the source are driest, when wind velocities tend to be highest, and when relative humidities in the plant stand are lowest.

During the 9-day sampling period under discussion the greatest spore dissemination occurred on 8, 9, and 10 May. These 3 days were meteorologically similar and numbers of spores trapped and diurnal variation in spore numbers was quite comparable. Each of these mornings was clear and dew had formed during the preceding nights. During the day the temperature rose regularly to a maximum at about 1300 or 1400 hours EST and then fell gradually. Winds followed a regular pattern, increasing in speed with time until mid-afternoon and then gradually subsiding. The trend of relative humidity also followed a regular pattern, which was the reverse of the trend of temperature -- the minimum relative humidity coming at the time of the maximum temperature. Low numbers of spores were trapped during May 11th and 12th. These 2 days were characterized by high relative humidity, extensive cloudiness, rain, fog, and drizzle.

The following suggestions may be of value in future experiments utilizing coated rods as trapping devices:

1. The rods used in this trial (1.6 mm in diameter) were very efficient traps but somewhat small for most convenient handling. Furthermore, the method of removing particles for observation and counting was more time consuming and liable to inconsistencies than seems necessary. It is suggested that a method employed by Gregory (1) be adopted for future trials. This consists of using a cylinder 0.5 cm in diameter. A 5/8 inch square of transparent cellu-

lose film is dipped into molten glycerine jelly (gelatin 1 gm, glycerine 7 gm, water 6 ml, phenol 1 percent), drained, and wound around the cylinder. After exposure the film is stripped off, mounted in glycerine jelly on a microscope slide, and covered with a 3/4-inch square cover glass.

2. In the present trial the trapping devices were not protected from rain. An incomplete and possibly distorted record was obtained because of rain showers during the latter portion of the trapping period. It is suggested that thin sheets of metal be supported horizontally above the trapping devices in such a manner as not to interfere with air flow. The "roof" should be of sufficient size to prevent rain from coming in contact with the traps. Such shelters are widely used to prevent rain from falling on horizontally exposed slides at many pollen trapping stations throughout the country.

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GROWTH RATES OF ORANGE SEEDLINGS IN THE PRESENCE AND ABSENCE OF  
PHYTOPHTHORA SPP. AT TWO LEVELS OF IRRIGATION AND TWO LEVELS  
OF SOIL NITROGEN<sup>1</sup>

L. H. Stolzy, P. W. Moore, L. J. Klotz, and T. A. DeWolfe<sup>2,3</sup>

Abstract

Damage to fibrous feeder roots of citrus by *Phytophthora citrophthora* and *P. parasitica* (citrus brown-rot fungi) has been associated with certain cultural practices in citrus orchards. Two cultural practices associated with a low density of feeder roots are large applications of soil nitrogen and irrigation.

Large closed containers of Vapam-fumigated sandy loam planted to Bessie sweet orange seedlings were used to study the effects of these two *Phytophthora* spp., soil nitrogen, and two levels of irrigation on citrus seedling responses.

The presence of *Phytophthora* in the soil significantly reduced length and weight of shoot growth as well as tree trunk circumferences. The effects of *Phytophthora* were much less on the fall flush of growth in the second growing year than on previous growth. Quantities of roots were also reduced significantly in soil columns containing the fungi.

Soils receiving nitrogen in the form of calcium nitrate did not significantly affect top or root growth of citrus seedlings when compared with plants growing in soil receiving no applications of soil nitrogen. Plants growing in soils with low nitrogen were given foliar applications of urea when leaf analysis indicated deficiency levels of nitrogen.

The two levels of irrigation, 9 cbs and 60 cbs, produced significant differences in weight of top growth during the second summer's growth period. The wet treatments (9 cbs) produced more total plant growth in both the presence and absence of the fungi than did the dry treatments.

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The presence of *Phytophthora citrophthora* and *P. parasitica* in most citrus orchards is highly probable (4). Klotz et al. (3) have shown in greenhouse studies that excess water and certain nitrogen fertilizers favor the parasitism of citrus feeder roots by *Phytophthora* spp. A management program for fertilization could be such that only foliage applications of nitrogen are used but the addition of irrigation water to the soil is necessary for most citrus production. The amount and method of applying water is of vital importance in the control of certain soil organisms. The extent to which a citrus grower can alter a management program to control soil organisms is limited by soil profile characteristics. Until recently recommendations to dry out citrus orchard soils had little meaning because only a quantitative method was available for measuring soil moisture. With modern soil moisture instruments it is now possible to measure the relative wetness or dryness of a soil *in situ*.

The purpose of this study was to determine the effects of two cultural practices on citrus root rot problems.

MATERIALS AND METHODS

Twenty concrete drain tiles 48 inches in length were used as soil-plant cultures (Fig. 1). Fourteen of these were 36 inches in diameter and six were 21 inches in diameter. The six smaller cylinders were equal to about the same volume as two of the larger ones and so were used as a substitute for two of the larger cylinders. The cylinders were buried in soil to a depth three fourths that of their respective lengths and were sealed on the inside and bottom with an asphalt compound. These were filled to a depth of 40 to 42 inches with a Tujunga fine

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<sup>1</sup> University of California Citrus Experiment Station Paper No. 1143.

<sup>2</sup> Respectively, Assistant Irrigation Engineer, Department of Soils and Plant Nutrition; Specialist, Department of Horticulture; Pathologist and Associate Specialist, Department of Plant Pathology, University of California, Riverside.

<sup>3</sup> In this study the authors gratefully acknowledge the assistance of T. E. Szuszkiewicz, a member of the Department of Soils and Plant Nutrition, University of California Agricultural Experiment Station.



FIGURE 1. Experimental area showing the cylinders filled with soil and planted with citrus seedlings. The uprights at each cylinder are mercury manometers for the tensiometers.

sandy loam. A Vapam drench at the rate of 800 pounds per acre was then applied to the soil in each cylinder (2). The following eight treatments were replicated two times in a randomized block experiment:

<u>Treatment No.</u>	<u>Treatment</u>	<u>Treatment No.</u>	<u>Treatment</u>
1	I <sub>1</sub> -Phy <sub>0</sub> -N <sub>f</sub>	5	I <sub>2</sub> -Phy <sub>0</sub> -N <sub>f</sub>
2	I <sub>1</sub> -Phy <sub>+</sub> -N <sub>f</sub>	6	I <sub>2</sub> -Phy <sub>+</sub> -N <sub>f</sub>
3	I <sub>1</sub> -Phy <sub>+</sub> -N <sub>s</sub>	7	I <sub>2</sub> -Phy <sub>+</sub> -N <sub>s</sub>
4	I <sub>1</sub> -Phy <sub>0</sub> -N <sub>s</sub>	8	I <sub>2</sub> -Phy <sub>0</sub> -N <sub>s</sub>

The explanation of treatment designations is as follows:

I<sub>1</sub> - Wet treatment (Irrigations at 9 centibars)

I<sub>2</sub> - Dry treatment (Irrigations at 60 centibars)

Phy<sub>0</sub> - Soils uninoculated

Phy<sub>+</sub> - Soils inoculated with Phytophthora spp.

N<sub>f</sub> - Foliage sprays of urea, no soil nitrogen added

N<sub>s</sub> - Nitrogen (Ca(NO<sub>3</sub>)<sub>2</sub>) added to soil at rates of 300 pounds/acre

Half of the number of cylinders were inoculated with zoospore suspensions of both a mixture of eight isolates of Phytophthora parasitica and a mixture of eight isolates of Phytophthora citrophthora. The inoculum was poured into 3-inch-deep dibble holes distributed over the soil surface in the cylinder. After the water of the suspension had drained into the soil the holes were filled with soil.

Bessie sweet orange seedlings were planted on November 29, 1956. Just prior to the planting the roots of the seedlings were heat treated in agitated water for 10 minutes at 112° F (1). Four seedlings were planted in each of the smaller cylinders. After the seedlings were established they were thinned so that there were three in each large cylinder and one in each small cylinder.

Two tensiometers were installed in each cylinder, one at a depth of 6 inches and the other at 18 inches. At the termination of the fall flush of growth for 1957 the 6-inch tensiometers were extended to the bottom of the cylinders. Prior to this the seedlings were irrigated according to suction values at the 6-inch soil depth. Following the relocation of the 6-inch tensiometers, the seedlings were irrigated according to suction values at the 18-inch soil depth. Differential irrigation treatments were started on August 9, 1957.

The length of shoots produced by each seedling was measured after the growth flushes had terminated. After the fall flush of 1958, the seedlings were pulled, fractionated, weighed, and trunk circumference measured. The soil in each cylinder was passed through a screen in order to measure quantities of roots per treatment.

## RESULTS AND DISCUSSION

Two of the three treatment factors that caused differences in seedling growth were the presence of *Phytophthora* spp. and the levels of irrigation. Nitrogen added to the soil in the form of calcium nitrate had no measurable effect on plant response when compared with plants receiving only foliage applications of nitrogen. These findings confirm the results obtained by Klotz et al. (3) in greenhouse studies in which citrus seedlings fertilized with calcium nitrate were less susceptible to attacks by root rot fungi than seedlings fertilized with other forms of nitrogen fertilizers.

Seasonal shoot growth measurements taken at the termination of each flush of growth for the fall of 1957, spring of 1958, and fall of 1958 are presented in Table 1. The most shoot

Table 1. Seasonal shoot growth, in centimeters, measured at the termination of each flush for two irrigation levels in the presence and absence of *Phytophthora* spp.

Treatment	Fall 1957			Spring 1958			Fall 1958		
	Phy <sub>0</sub>	Phy <sub>+</sub>	Total	Phy <sub>0</sub>	Phy <sub>+</sub>	Total	Phy <sub>0</sub>	Phy <sub>+</sub>	Total
I <sub>1</sub>	5821	4330	10151	8770	5987	14757	19759	20590	40349
I <sub>2</sub>	5202	4362	9564	7330	6816	14146	18420	17779	36199
Total	11023*	8692		16100*	12803		38179	38369	

\*Significant difference at 5 percent level between citrus seedlings growing in soils with and without *Phytophthora* spp.

growth during the first period was produced by the wet treatment (I<sub>1</sub>) in the absence of *Phytophthora* spp. The presence of the fungi significantly reduced the seedling shoot growth for this same period. The small apparent differences in shoot growth for the two irrigation levels were due to the fact that differential irrigation treatments were not started until late in the summer season of 1957. The same measured effects from treatments were present in the shoot growth data for the spring of 1958 as were present in the shoot growth data for the fall of 1957. The presence of *Phytophthora* spp. significantly reduced shoot growth while the level of irrigation had little effect. The least shoot growth for the spring period, however, was obtained from seedlings in the wet treatment with the fungi present. During this time of the year soil temperatures keep root activities at a minimum but favor the growth of certain *Phytophthora* spp. The shoot growth for the summer and fall of 1958 showed no significant differences for the treatment combinations in Table 1. The wet treatment (I<sub>1</sub>) produced the most total shoot growth for this period. A secondary analysis of these data (fall, 1958) showed a significantly higher shoot growth for the seedlings in treatment 2(I<sub>1</sub>-Phy<sub>+</sub>-N<sub>f</sub>) than for the seedlings in treatment 6(I<sub>2</sub>-Phy<sub>+</sub>-N<sub>f</sub>). The presence of soil nitrogen in treatment 3(I<sub>1</sub>-Phy<sub>+</sub>-N<sub>s</sub>) and 7(I<sub>2</sub>-Phy<sub>+</sub>-N<sub>s</sub>) caused an unexplained interaction between the wet and dry treatments.

The experiment was terminated in the fall of 1958. At this time the seedlings were separated into two periods, one including the growth of 1957 and spring 1958 and the other the summer and fall growth of 1958. Fresh weights for the two periods were then obtained. The shoot growth for the summer and fall of 1958 was separated from the rest of the aerial portion of each seedling. Cross-sectional increases in the older portions of the seedlings during the summer and fall of 1958 would account for some of the weight recorded for the early growth period. Analysis of the data in Table 2 showed the same general trends as the analysis in Table 1. Differential irrigations had little effect during the cooler seasons while the presence of *Phytophthora* spp. caused highly significant differences in citrus seedling weights for the period including all of 1957 and spring of 1958. Seedlings from the dry treatments (I<sub>2</sub>) in the presence of soil fungi produced the least amount of growth. During the summer and fall seasons of 1958, seedlings from the wet treatment increased significantly more in weight than those from the dry treatments (Table 2). The presence of *Phytophthora* spp. in the wet treatment had no effect on the amount of aerial growth during this period. The effect of *Phytophthora* spp. in the dry treatments was very apparent.

The fresh weights of citrus roots from each treatment are given in Table 3. Roots were collected and weighed in two separate lots above and below the 18-inch soil depth. This was the

Table 2. Weight of aerial portions, in grams, of citrus seedlings for two periods, grown at two irrigation levels in the presence and absence of Phytophthora spp.

Treatment	: 1957 and Spring Flush 1958			: Summer and Fall Growth 1958		
	Phy <sub>0</sub>	Phy <sub>+</sub>	Total	Phy <sub>0</sub>	Phy <sub>+</sub>	Total
I <sub>1</sub>	13793	10140	23933	11150	10959	22109***
I <sub>2</sub>	13463	9062	22525	8954	6913	15867
Total	27256***	19202		20104	17872	

\*\*\*Significant difference at 0.1 percent level between seedlings irrigated at two different levels and also between seedlings growing in soils with and without Phytophthora spp.

Table 3. Fresh root weight, in grams, of citrus seedlings for two irrigation levels in the presence and absence of Phytophthora spp.

Treatment	: 0 to 18 inch soil depth			: 18 to 40 inch soil depth		
	Phy <sub>0</sub>	Phy <sub>+</sub>	Total	Phy <sub>0</sub>	Phy <sub>+</sub>	Total
I <sub>1</sub>	12114	8179	20293	4878	3648	8526
I <sub>2</sub>	10447	6504	16951	4494	3606	8100
Total	22561**	14683		9372	7254	

\*\*Significant difference at 1 percent level between quantities of roots of seedlings growing in soils with and without Phytophthora spp.

depth at which irrigation levels were determined. The soil horizon from 0 to 18 inches had a higher average suction than the soil horizon from 18 to 40 inches except for short periods following irrigations. The presence of Phytophthora spp. significantly reduced concentration of roots in the 0 to 18 inch soil depth. The dry treatment (I<sub>2</sub>) with the fungi present reduced the root concentration more than any of the other treatment combinations in both the 0 to 18 and 18 to 40 inch soil depths. The lack of significance in fresh root weights for the 18 to 40 inch depth could have resulted from two factors: (a) since the inoculum was placed in the surface soil, the fungi were slow in developing at the deeper depths, (b) the soil moisture environment could have been more favorable in both the wet and dry treatments at the greater depth.

Trunk circumference measurements are given in Table 4. Again the presence of Phytophthora spp. significantly reduced growth in trunk size of seedlings. Smallest trunk circumfer-

Table 4. Average trunk circumference, in centimeters, of citrus seedlings grown at two irrigation levels in the presence and absence of Phytophthora spp.

Treatment	Phy <sub>0</sub>	Phy <sub>+</sub>	Average
I <sub>1</sub>	12.5	11.0	11.8
I <sub>2</sub>	12.1	10.5	11.3
Average	12.3***	10.8	

\*\*\*Significant difference at 0.1 percent level between seedlings growing in soils with and without Phytophthora spp.

ences of seedlings were from dry treatments with the fungi present. This study showed that measured plant responses were generally greater in the wet treatments (I<sub>1</sub>) than in the dry treatments (I<sub>2</sub>). This trend was evident in the presence and absence of Phytophthora spp. This indicates that the practice of drying out orchard soils to reduce root rot problems is unnecessary unless excess water has been added to the soil either from rains or irrigations. In the case of irrigation water it is far more important to avoid adding excessive amounts of water to the soil. It is also important to avoid premature irrigations in the spring when roots are inactive. A recommended practice for many citrus orchards would be irrigations of shorter duration with the frequency of irrigations adjusted to the season of the year based on instrument readings.

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39. 24

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LABORATORY METHOD FOR TESTING EFFECTIVENESS OF SOIL DISINFESTANTS

L. J. Klotz, T. A. DeWolfe and R. C. Baines

For determining the fungicidal value of liquids and water soluble chemicals at various depths in the soil, a rapid laboratory method was developed. In lucite cylinders measuring 42 inches in length and 4 inches in diameter, rectangular slots 1 inch wide and 3 inches long were cut at three levels 12 inches apart by use of a hot scalpel to make the initial small cuts and a hack saw blade to saw out the rectangular pieces. Soil and citrus seedlings, whose roots were inoculated with *Phytophthora parasitica*, were added to the cylinders in the following manner. The open-ended cylinders were placed in the container and held in a vertical position as shown in Figure 1. Loamy top soil, with 10 percent moisture for ease of handling and keeping the citrus seedlings alive, was added so that after gentle tamping the soil was 2 inches above the bottom of the lowest rectangular slot. A citrus seedling whose root system had been dipped in a zoospore suspension of *P. parasitica* was then planted in the soil so that the seedling top protruded from the cylinder. This process was repeated until inoculated seedlings were at the three levels shown in Figure 1 and Table 1.

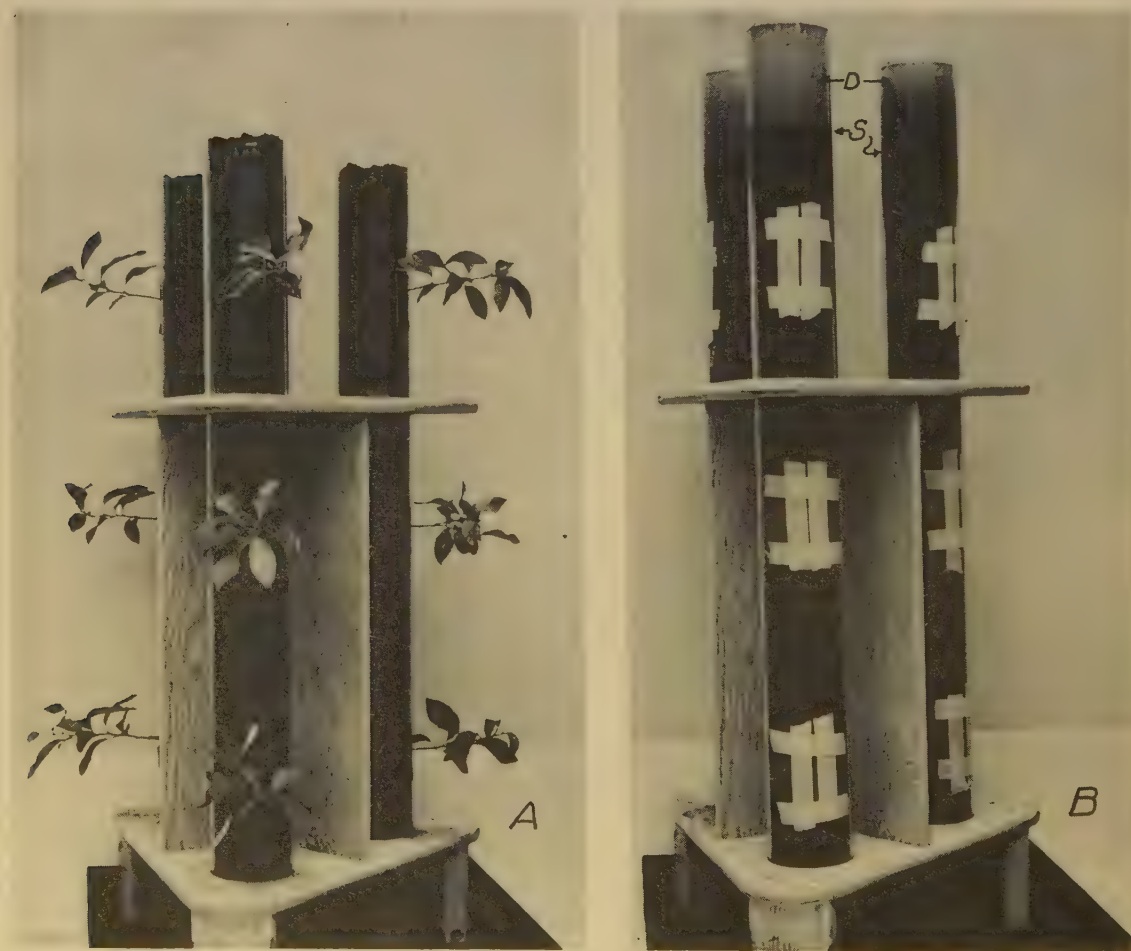


FIGURE 1. Equipment for testing soil disinfestants: A -- lucite cylinders (42 inches in length, 4 inches in diameter) with inoculated seedlings planted in infested soil through rectangular holes (3 inches x 1 inch) in lucite; B -- tops of plants cut off and holes sealed prior to adding disinfestants (D) to soil surface (S).

Table 1. Fungicidal effectiveness of two water-soluble compounds in soil.

Test number <sup>a</sup>	Depth (in inches)	Material A	Material B	Water (control)
2	6	- <sup>b</sup>	-	+
1	12	-	-	+
2	18	-	-	+
1	24	-	+	+
2	30	-	+	+
1	36	+ <sup>b</sup>	+	+

<sup>a</sup> This tabulation includes the results of two experiments.

Koethen sweet orange seedlings were used in the first test and Eureka lemon in the second. The depths of placement of the infected seedlings are shown in the second column.

<sup>b</sup> + = *P. parasitica* recovered from citrus seedlings and soil;

- = fungus not recovered and presumably killed by disinfestants.

After 10 days were allowed for the roots to become thoroughly invaded by the fungus, the tops of the seedlings were cut off and the rectangular pieces of lucite replaced in the slots and sealed with adhesive tape. A powdered material (A) used at the rate of 400 pounds per acre was mixed with the surface inch of soil of one cylinder and water added to a depth of 6 inches. A liquid material (B) used at the rate of 400 pounds per acre was placed in a 30-ml beaker and set on the soil surface of another cylinder. Water to provide a depth of 6 inches was poured into the beaker containing the chemical B and allowed to overflow onto the soil. The control cylinder received only water.

Two weeks later the seedling roots and soil at the three levels were removed and cultured for *Phytophthora parasitica*, using the apple and lemon techniques<sup>1</sup>.

The results in Table 1 are from two tests, the upper soil surface being at 12 inches above the top seedling in the first test and at 6 inches in the second test. Thus data were secured at 6-inch intervals of the soil columns.

Obviously the method is usable with any liquid or soluble material and with other test plants, fungi, and nematodes. The rate of percolation of the liquids and phytotoxicity to the host plants are also observable.

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<sup>1</sup> Klotz, L. J., and T. A. DeWolfe. 1958. Techniques for isolating *Phytophthora* species which attack citrus. Plant Disease Repr. 42: 675-676.

PRELIMINARY REPORT ON THE SYSTEMIC ACTIVITY OF CAPTAN  
WHEN APPLIED TO THE SOIL FOR THE CONTROL OF BLACK SPOT OF ROSES<sup>1</sup>

*not done  
as early as possible  
2 1/2 weeks later* H. R. Rosen<sup>2</sup>

In a previous article (2) attention was directed to the need of fungicides with systemic properties for adequate control of black spot (*Diplocarpon rosae*) of roses. It was noted that unlike most members of the rose family, modern garden roses possess an indeterminate type growth and a continuity of blooming throughout the growing season. Because of this, new growth can be expected after a foliar application of a fungicidal spray or dust and this growth will be unprotected unless the fungicide used possesses some systemic properties. It is also obvious that the longer the period of time between such applications the greater will be the amount of unprotected growth.

Following the excellent work of May, Palmer and Haeskeylo (1) in which they found fungicidal properties in foliar extracts of different species of plants grown in soil impregnated with captan (N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide), an experiment was undertaken to determine the effectiveness of this material in the control of black spot and other foliar diseases of roses when it is applied to the soil. May et al. had included roses among their test plants and had used the Dutch elm disease organism, *Ceratocystis ulmi*, seeded on agar plates as a tester for fungicidal properties because of its sensitivity to captan. Would this material applied to the soil protect rose bushes against black spot?

The first experiment consisted of three replicates of as many susceptible varieties, using five plants per replicate, and checked against untreated plants and plants sprayed or dusted with a number of other fungicides. Since captan had already been proved a good fungicide for the control of black spot when applied as a foliar spray, it was not used as a spray in these tests.

The first soil application was made on April 24, after a sufficient amount of infection and inoculum had developed in the plots as a whole to permit later uniformity of infection. The amount of captan used was 5 pounds of 50 percent wettable powder per 25 square feet. It was applied by hand as uniformly as possible around each plant and worked lightly into the top soil with a garden hoe.

Since there was some slight indication that one soil application might not be sufficient to protect the plants throughout the growing season, a second soil application was made on May 24 using the same amount of captan applied in the same way on the same plots.

In the meantime abundant rains had fallen after the first application so that there was ample opportunity for the fungicide to be carried to a considerable part of the root systems and also to permit numerous black spot infections. Likewise, there were abundant rains immediately after the second application. The root systems in these plots were derived from two types of understock, *Rosa multiflora* and Ragged Robin (*Gloire des Rosomanes*). There seemed to be no marked difference between them relative to their reaction to captan soil treatment.

The plots were examined frequently to note any possible injury from the soil treatments as well as to attempt to measure amounts of foliar infections. Because of the rapidity of defoliation caused at first by black spot infections, accurate counts of numbers of infections were abandoned in favor of comparative or relative amounts. When any question arose as to ratings, the plants were reexamined and rerated several times at any one reading.

As the season advanced reading on the relative amount of black spot infection and particularly on defoliation due to black spot became complicated by increased abundance of anthracnose lesions (*Elsinoë rosarum*) and by drought injury.

Within a few weeks after the first soil treatment with captan it was clearly obvious that there was considerably less black spot on these plots than on untreated plots and even less than on most plots that had received weekly sprays or dusts with such fungicides as Phaltan, maneb, Acti-dione PM, semicarbazone, Cyprax and COCS.

An indication of the control of black spot obtained by the soil treatment may be seen in the data on amounts of black spot estimated on May 28, shown in Table 1.

The trace of black spot noted on plants with captan soil treatment probably represented infections that occurred prior to the application of the fungicide. No black spot was found on the newer growth and no additional black spot was found on these until early July. By that time the untreated controls were 90 percent defoliated, resulting mostly from black spot infections, while many of the plots with weekly applications of sprays or dusts also showed con-

<sup>1</sup> Submitted with the approval of the Director of the University of Arkansas Agricultural Experiment Station.

<sup>2</sup> Emeritus Professor of Plant Pathology, University of Arkansas, Fayetteville, Arkansas.

Table 1. Comparative amounts of black spot with different fungicidal treatments.

Variety	Type of treatment	Relative percent of black spot
Peace	Captan on soil	trace
Peace	Weekly orchard oil spray	10
Aztec	Captan on soil	trace
Pink Peace	Captan on soil	trace
Pink Peace	Weekly Acti-dione PM spray	5
Green Fire	Untreated control	30
Ruby Lips	Untreated control	30

siderable defoliation from black spot (Fig. 1). In contrast, the three plots that had received the captan soil treatment showed little defoliation up to the middle of July and what little there was appeared to be due to anthracnose infections and drought injury.



FIGURE 1. Three plants at left with captan soil treatment, three plants at right with Acti-dione PM weekly spray. Photographed July 13, 1959. All plants shown are of the variety Pink Peace.

While there appears to be no doubt that captan applied to the soil in the amount utilized acted as a systemic fungicide for the control of black spot, captan apparently did not control powdery mildew or anthracnose.

Unlike black spot, powdery mildew (*Sphaerotheca pannosa* var. *rosae*) was quite spotty throughout the plots; some plants had a considerable amount, others only slight amounts and still others had none, even with plants of the same variety. In the plots with captan soil treatment only a few plants showed any powdery mildew, but these few were enough to show that no control was obtained. It is of interest to note that this fungicide applied as a foliar spray does not control this disease -- as a number of investigators have found -- and it behaves the same way when it or a derivative of it is present within the plant.

Anthracnose, like powdery mildew, was not controlled in those plots with captan soil treatment, at least not nearly so well as black spot. In 1959 anthracnose in considerable amounts appeared later than black spot, but it caused much defoliation and was not adequately controlled by any of the fungicides used, with the possible exception of COCS used as a weekly spray.

In conclusion, it should be clearly understood that this was merely a preliminary experiment, the results of which require much duplication over a series of years and in different parts of the country. Even if other workers should obtain similar results, there are still many questions remaining to be answered. These include: the best time and amount of application; possible deleterious effects on the plants when soil treatments are given on the same plots over a period of years; and trials of other fungicides for systemic properties.

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ROOT ROT OF ALFALFA CAUSED BY PHYTOPHTHORA CRYPTOGEA IN ILLINOIS<sup>1</sup>J. W. Bushong and J. W. Gerdemann<sup>2</sup>Summary

A highly destructive root rot disease of alfalfa has been found in Illinois. Five isolates of Phytophthora from alfalfa in Illinois were compared with an isolate of P. cryptogea obtained from alfalfa in California. Four of the Illinois isolates were nearly identical with the California isolate in both morphology and pathogenicity. One Illinois isolate was morphologically distinct and differed in pathogenicity from the other isolates.

Root rot of alfalfa, Medicago sativa, caused by Phytophthora cryptogea Pethy. & Laff., was first described by Erwin (2) in 1953. Since then Erwin (3, 4), and Erwin and Kennedy (5) have published detailed studies of the disease. At present this disease has been reported only from California. During the spring of 1957, a similar alfalfa root rot was found in seven fields in Illinois. Since 1957, it has been found in three additional fields. An apparently identical disease was previously observed in September 1951 in a field in Jefferson County, Illinois. Although this disease is not yet common in Illinois, it is widely distributed (Fig. 1) and has been highly destructive in individual fields. The disease has been observed on heavy soils with poor drainage (Fig. 2) and appears to be favored by cool, rainy weather and standing water. It has been found in first or second year stands of Buffalo, Ranger, Kansas Common, and Dupuits varieties of alfalfa. Several of the fields in which the disease occurred had been repeatedly cropped to alfalfa.

## SYMPTOMS

When conditions are favorable for the disease, the symptoms develop rapidly and plants wilt and die with little or no stunting. When conditions are less favorable (if the weather becomes warm and dry following infection), the progress of the disease is less rapid. The plants become stunted, chlorotic, and die much more slowly. The above-ground symptoms are accompanied by a soft rot of the crown and tap root. The periderm and vascular tissues of the tap root show a reddish-brown necrotic area with a yellowish discoloration extending above and below the lesion. When decay is near the crown, attempts to pull the root out of the soil often result in the crown separating from the tap root.

## ISOLATION

Alfalfa roots from which tissue was to be plated were first washed in tap water. Sections 1 to 1 1/2 inches long containing the yellowish advancing margin of decay as well as the adjoining white tissue were cut from these roots, immersed in a 10 percent solution of "Clorox" (5.25 percent sodium hypochlorite) for 20 to 30 seconds and rinsed in sterile distilled water. The pieces of roots were split longitudinally and 3 to 10-mm sections were removed aseptically from the advancing margin of decay in the xylem and phloem. This tissue was placed on Difco corn meal agar in Petri dishes and incubated at room temperature. Hyphal tip transfers were made after 3 to 5 days. Isolates of Phytophthora were frequently obtained from diseased roots during early spring and fall; however, only Fusarium spp. and some other unidentified fungi and bacteria were isolated during the summer.

Five isolates of Phytophthora obtained from alfalfa in Illinois were compared with an isolate of P. cryptogea which had been isolated by Erwin from alfalfa in California. For convenience, the Illinois isolates are designated by the name of the town nearest to the field from which they were obtained; for example, Lincoln, Melvin, Chrisman, Alvin, and Kankakee.

<sup>1</sup> A portion of a thesis submitted by the senior author to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Science.

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FIGURE 1. Distribution of *Phytophthora* root rot of alfalfa in Illinois. Each spot represents a field with diseased plants.

#### MORPHOLOGY

The Melvin, Chrisman, Alvin, and Kankakee isolates of *Phytophthora* appeared morphologically indistinguishable from the California isolate. Mycelial growth was typical for the genus, exhibiting characteristic right-angle branching with a slight constriction at the base of each branch, as noted by Blackwell (1). Hyphae ranged from 3 to 8  $\mu$  in diameter and averaged 6  $\mu$  in diameter. In young cultures hyphae were non-septate but became septate with age. Numerous intercalary and terminal hyphal swellings developed when grown upon corn meal agar or hemp seeds in water. They usually formed in clusters, were often irregular in shape, and were not delimited by septa. The swellings were typical of those of *P. cryptogea* as illustrated by Erwin (3). Oogonia were formed in abundance after 3 to 5 days. They were typically globose



FIGURE 2. Pattern in diseased alfalfa field with concentration of Phytophthora-infected plants in low spots.

and usually filled by the smooth, globose oospores. The type of antheridial attachment was exclusively amphigynous. Sporangia formed sparsely on hemp seed in water in culture tubes; however, when 1-week-old cultures were poured into Petri dishes, numerous sporangia frequently appeared in 7-10 days. Sporangia were non-papillate and typically ovoid to obpyriform in shape. They often germinated by the formation of a germ tube which grew from the tip or side of the apex. Proliferation was common. The isolates did not differ significantly in size of sporangia, oogonia, oospores and oospore walls. The measurements were similar to those reported by Erwin (3).

The Lincoln isolate differed from the others in that it had both the amphigynous and paragynous type of antheridial attachment, with the paragynous type predominating. Also, hyphal swellings were not formed and the oospore wall was thicker. Sporangia were not produced on hemp seed in water, but did form in non-sterile pond water. These sporangia were decidedly smaller than those produced by the other isolates.

#### TEMPERATURE-GROWTH RELATIONSHIP

The cardinal temperatures were determined for all isolates on corn meal agar at 5°, 10°, 15°, 20°, 25°, 30°, and 35°C. The minimum, maximum and optimum temperatures for the Melvin, Chrisman, Kankakee, and California isolates were essentially the same. The optimum was 25°. They grew only slightly at 5° and made no growth at 35°. The Lincoln isolate consistently grew at both 5° and 35°, with the optimum at 25°C.

#### PATHOGENICITY

The Phytophthora isolates were tested for their ability to cause damping-off of alfalfa. The fungi were grown in Erlenmeyer flasks containing hemp seed and water at room temperature for 2 weeks. The mycelium was transferred to sterile deionized water in a Waring Blendor and ground for 2 to 3 seconds. Alfalfa seeds were placed on the surface of steam-sterilized soil in pots and 10 ml of mycelial suspension was poured over them. The seeds were covered with 1/2 inch layer of sterilized soil and watered with distilled water. Two types of checks were used in which 1) distilled water, and 2) water from hemp seed media were substituted for the inoculum. The experiment was conducted at 15.5° to 21°C and repeated at 21° to 26.5°C. There were five pots of 50 seeds each for each isolate at each temperature. The emergence was greatly reduced in infested pots and in most cases a high percent of the emerging plants were killed by post-emergence damping-off. (Table 1).

Pre-emergence damping-off was most severe at the lower temperatures. At the higher temperatures, the Lincoln isolate caused less post-emergence damping-off than did the other isolates. The seedlings in the check pots remained healthy.

The pathogenicity of the Illinois and California Phytophthora isolates was tested on 6-month-old alfalfa plants that had been grown from seed in sterilized soil. Prior to inoculation all top growth was cut back to 6 to 8 inches. One inch of soil was dug away from the tap roots and a wound 1/2 inch long was made to the center of each root slightly below the crown. A bit of mycelium from a 10-day-old culture was inserted into the wound and the soil immediately replaced around the roots. Wounds were made in the check roots without the introduction of mycelium. Twenty plants were used for each isolate. The plants were watered from below to prevent splashing. The soil was kept moist and the temperature remained fairly constant at approximately 21°C during the experiment.

Table 1. Emergence and damping-off of alfalfa seedlings in soil infested with five isolates of *Phytophthora* at two different temperatures.

Isolate	15.5° to 21° C		21° to 26.5° C	
	Total plants	Total plants	Total plants	Total plants
	emerged	killed by post-emergence damping-off	emerged	killed by post-emergence damping-off
	:	:	:	:
Lincoln	8	2	47	11
Melvin	0	0	46	42
Kankakee	13	11	74	50
Chrisman	1	1	47	40
California	12	11	58	47
Distilled water check	212	0	207	0
Hemp seed water check	222	0	201	0

Table 2. Incidence of disease in 6-month-old alfalfa plants inoculated with five *Phytophthora* isolates.

Isolate	Number of plants with:			
	slightly to moderately	severely decayed	number of plants dead	
	no decay on roots	decayed roots	roots	and with completely decayed roots
	:	:	:	:
Lincoln	15	5	0	0
Melvin	2	9	0	9
Kankakee	1	3	1	15
Chrisman	0	4	6	10
California	2	12	0	6
Check	20	0	0	0

Table 3. Rotting of apples and potatoes inoculated with *Phytophthora* isolates from alfalfa.

Isolate	Extent and type of decay in:	
	apple	potato
Lincoln	complete soft brown	complete soft pink
Melvin	none	none
Kankakee	none	none
Chrisman	none	none
California	incomplete soft brown	none

After 4 days, some plants inoculated with the Kankakee, Melvin, Chrisman, and California isolates showed moderate wilting. At the end of 2 weeks, many plants were severely wilted, chlorotic and dying (Fig. 3). The results of the experiment were recorded 3 weeks after inoculation (Table 2).

Three Illinois isolates and the California isolate were highly pathogenic; however, the California isolate was slightly less virulent. The Lincoln isolate, which is morphologically distinct from the others, was only slightly pathogenic. The checks remained healthy with only a slight discoloration around the wound.

In severely wilted plants the roots had a reddish-brown to dark brown necrotic area extending approximately 1 1/2 inches below the crown and completely encircling the root. Where rotting was extensive, the necrotic area had a stringy appearance, the host tissues were very soft and water-soaked, and the root separated easily from the crown. A yellowish discoloration in the xylem extended approximately 1/2 inch above and below the lesion. These symptoms appeared identical with those observed on naturally infected plants. The causal fungus was consistently reisolated from the diseased roots.



FIGURE 3. Ranger alfalfa plants 2 weeks old after wound inoculation with an Illinois *Phytophthora* isolate from alfalfa. Left -- inoculated. Right -- check. Top growth of plants in both pots was cut back at the time of inoculation.

Erwin reported that *P. cryptogea* from alfalfa was only slightly pathogenic on apple and non-pathogenic on potato (3). Idaho potato tubers and ripe Jonathan apples were inoculated with the California isolate and the four Illinois isolates from alfalfa. A cut was made in the apples and potatoes with a sterilized scalpel, and a bit of mycelium from 1-week-old cultures growing on hemp seed in water was inserted into the wound. The wound was covered with petroleum jelly. Check apples and potatoes were cut and the wound covered with petroleum jelly. The experiment was incubated at room temperature and concluded after 10 days (Table 3).

The California isolate rotted apples slightly, thus differing from three of the Illinois isolates which caused no rotting on apples. The Lincoln isolate differed strikingly from the other four by rotting apples and potatoes completely.

#### DISCUSSION

The Alvin, Melvin, Kankakee, and Chrisman isolates are nearly identical with the California isolate of *P. cryptogea* and it is concluded that they should be referred to this species. The Lincoln isolate is distinctly different. A combination of growth at 35°C and predominantly paragynous antheridia appears to separate this fungus from previously described species of *Phytophthora* if one uses Tucker's (6) system of classification. However, it has been isolated only once and since it was shown to be only weakly pathogenic to alfalfa, it is probably of minor importance as a root rot pathogen of this crop.

This disease is not yet common in Illinois; however, it is widely distributed and has caused serious losses in individual fields. This disease looms as a potential threat to alfalfa production especially on poorly drained, heavy soils.

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THE DIFFERENTIATION OF PATHOGENIC FORMS  
OF EU-HELMINTHOSPORIUM SPECIES WHICH ATTACK OATS<sup>1</sup>

R. W. Earhart<sup>2</sup>

Abstract

Mycelial macerates of certain Eu-helminthosporium species yield water-soluble substances toxic to oat seedlings. A simple laboratory procedure which differentiates between pathogenic forms of these fungi was used to determine the presence and prevalence of these forms in South Carolina. Host responses to these toxic materials can be determined within 24 hours, and responses to mycelial macerates and to toxin filtrates are essentially the same. Measurements of two symptom expressions by four oat varieties indicated that two groups of these fungi were represented among the isolates evaluated. The largest group, comprising 94.4 percent of all isolates, includes the forms which incite Victoria blight; the other and much smaller group includes those forms which incite the culm rot disease of Southland oats.

INTRODUCTION AND LITERATURE REVIEW

Briosi and Cavara in 1889 described the first Helminthosporium with demonstrated pathogenicity upon oats. This species, H. avenae, is an important pathogen of cultivated oats, and causes large losses on this crop every year. In 1928, Nishikado (8) placed H. avenae in the subgenus "Cylindro-helminthosporium" because it has cylindrical conidia which germinate from both polar and intercalary cells, with its ascigeral stages in Pyrenophora or Pleospora. The Eu-helminthosporium group, in contrast, has long elliptical, obclavate fusiform conidia more or less tapered towards both ends, and germinating only from the polar cells. This group of fungi has Ophiobolus (Cochliobolus) ascigeral stages.

Helminthosporium avenae was the only recognized oat pathogen within the genus until 1946. In that year, Meehan and Murphy (6) described the new species H. victoriae, which was capable of blighting those oat varieties that have the gene which induces a necrotic reaction to certain races of crown rust from the variety Victoria. In 1951, Earhart (1) reported a Helminthosporium species which was capable of attacking oat varieties previously classified as resistant to H. victoriae.

Meehan and Murphy (7) demonstrated the role of the water-soluble toxins produced by H. victoriae in the etiology of the blight disease. Litzenberger (4) in 1949 described the use of these toxin-containing fungal materials for evaluating the pathogenicity of H. victoriae. Ivanoff (3) first used a mass screening method to select plants having apparent resistance to H. victoriae. Wheeler and Luke (11) in 1954 reported the isolation of the toxin "Victorin" produced by H. victoriae. These same workers (12, 5) developed a mass screening technique for oat seedlings that employed this toxin. From their research has come much of the practical knowledge on toxin production by these fungi. In 1957, Earhart (2) reported results from laboratory studies comparing the reactions of two oat varieties to the toxins produced by filtering, acidifying, and autoclaving mycelial macerates, and to the mycelial macerates from the same isolates. Pringle and Braun (10) isolated and partially purified the toxin elaborated by H. victoriae and in 1958 Pringle (9) described some of its chemical properties.

This paper describes a simple laboratory bioassay for determining the presence and prevalence of the pathogenic forms of some Eu-helminthosporium species capable of attacking oats. This technique uses the water-soluble toxins from mycelial macerates to measure their pathogenicity to four oat varieties. The method permits the rapid and reproducible determination of host responses to specific isolates, it requires only simple laboratory equipment, and it is easily learned by laboratory personnel.

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## MATERIALS AND METHODS

In December of 1955 approximately 500 fungal cultures were obtained from the subcrown areas of infected and healthy oat plants as well as the soil from oat fields in South Carolina. Among these cultures were over 300 from the genus Helminthosporium. Single conidial isolates were made from the Helminthosporium cultures for further study. In 1957 and 1958, 54 of these isolates, which, according to Nishikado's classification, would be grouped into "Eu-helminthosporium," were evaluated for their pathogenicity to four oat varieties. These were Victor-grain 48-93 and Arlington which have been considered moderately susceptible to H. victoriae and to possess the typical Victoria resistance to certain races of crown rust, and Midsouth and Southland which have been considered resistant to Victoria blight and lack resistance to these races of crown rust. The isolates used were selected at random, and no previous attempt had been made to determine their pathogenicity prior to entry in this test.

The fungus isolates were grown in Petri dishes on standard V-8 juice agar medium adjusted to pH 5.5 with calcium carbonate. Previous work had shown a minimum amount of saltation or other vegetative changes under these conditions.

Suspensions of these isolates were prepared by macerating the contents of two Petri dishes containing 10-day-old cultures in 100 ml of tap water for 60 seconds in a Waring Blendor, then diluting to 400 ml with additional tap water. This suspension was distributed by 5-ml aliquots to eighty 20-ml test tubes contained in four 24-tube racks. The four additional tubes in each rack were controls with two containing 5 ml of tap water and the other two containing 5 ml of sterile V-8 juice agar macerate and diluted in the same manner as the fungal suspensions.

The oat seedlings used as indicator plants were grown in sand in 6-inch clay pots. Cleaned and treated<sup>3</sup> seeds were planted at 1-inch depth, 30 seeds per pot. A uniform planting depth was used so that seedlings would be at a uniform stage of development for assaying pathogenicity.

Ten-day-old seedlings were washed free of sand and their roots were placed in tap water until the plants were turgid. One seedling was placed in each test tube, and a glass stirring rod was used to submerge the roots in the liquid.

The racks of test tubes containing the seedlings were placed under two 300-watt Mazda lamps, with the top of the test tube rack 11 inches below the lamps and all test tubes approximately the same distance from the light source. Under these conditions the seedlings received from 1200 to 1350 foot-candles of light. The heat from the lights resulted in a temperature of about 30°C around the plants regardless of the ambient room temperature. The 5 ml of liquid was sufficient for normal plant metabolism during the course of the test.

After 24 hours, the oat seedlings were classified individually according to the following symptom-expression categories: 1) rotted -- local necrosis of the culm and leaf sheaths due to action of the fungus; 2) blighted -- death of the leaf blades, generally accompanied by a blue-grey darkening of the tissues; 3) yellowed -- chlorosis, either with or without veinal areas showing chlorosis; 4) color change -- any leaf blade color other than green, blighted, or chlorotic; 5) tip burn -- a marginal blighting of the tip of the leaf blades, generally being a light brown in color; and 6) unaffected -- no apparent macroscopic change of leaf blade tissues. All symptom evaluations were made in comparison with the seedlings growing in the tap water and agar macerate blanks which served as controls.

## RESULTS AND DISCUSSION

The pathogenicity of 54 Eu-helminthosporium isolates was evaluated by this method. Table 1 presents a summary of two symptom expressions for six representative isolates. These six were selected as representative since the remaining 48 isolates could all be grouped into the six categories represented by those presented.

A considerable variation in symptom expressions was observed. The data presented in Table 1 summarize these symptom expressions according to the two symptoms "rotting and blighting," which are considered to be the most virulent expressions incited by these organisms.

The expressions of the two symptoms by the four host varieties can be used to group these six fungal isolates according to their relative ability to incite these symptoms. Such groupings show that two natural categories are represented by these six isolates. The first is represented by isolate No. 16, which was characteristic of only 3 of the 54 isolates evaluated. These three isolates were capable of blighting Southland, but no other variety. It is believed that these

<sup>3</sup> Seed was cleaned by fanning and screening, then treated with the chemical fungicide, 2.25 percent methyl mercury 8-hydroxyquinolate, at the rate of 3/4 ounces per bushel of seed.

Table 1. Reactions of four oat varieties to six South Carolina isolates of Eu-helminthosporium species.

Isolate number	Symptom expression	Percent seedlings affected <sup>a</sup>			
		: Victorgrain 48-93	: Midsouth	: Arlington	: Southland
22	Rotting	0.0	0.0	0.0	0.0
	Blighting	1.2	0.0	0.0	0.0
27	Rotting	0.0	0.0	0.0	0.0
	Blighting	47.5	37.5	22.5	0.0
1	Rotting	0.0	0.0	0.0	0.0
	Blighting	26.2	26.2	18.7	27.5
11	Rotting	0.0	0.0	0.0	0.0
	Blighting	60.0	100.0	50.0	20.0
15	Rotting	31.2	13.7	6.2	23.7
	Blighting	5.0	33.7	25.0	3.7
16	Rotting	0.0	0.0	0.0	0.0
	Blighting	0.0	15.0	5.0	100.0

<sup>a</sup> Percent of seedlings from four independent tests of 20 seedlings each which manifested these symptoms.

isolates represent the members of the genus Helminthosporium which are commonly grouped together under the name "culm rot incitants." Culm rot was first described on the variety Southland in 1951 (1). The other five isolates, which are representative of the other 51 of the 54 isolates evaluated, exhibit a more varied pathogenic pattern.

This second group includes all those Helminthosporium isolates which are now included in the species H. victoriae, since they were pathogenic to those oat varieties which have a gene for crown-rust resistance derived from the variety Victoria. However, also in this group were some isolates that were "not too typical," although pathogenically similar to the described species. Therefore, instead of a single homologous group these isolates may represent closely allied groups of fungi.

Among these fungi was the innocuous isolate No. 22 (characteristic of 2 of the 54 isolates evaluated) which had little apparent pathogenicity. Also isolate No. 27 (characteristic of 23 of the 54 isolates) which represented a moderately virulent fungus which behaved as a "true" H. victoriae isolate with no demonstrable pathogenicity to Southland. Then two isolates, No. 1 (characteristic of 6) and No. 11 (characteristic of 16 isolates), which represent two levels of pathogenicity of a fungal form which is not fully understood, but which may represent an intermediate stage between the "culm rot" and the "Victoria blights." These isolates were not only able to attack oat varieties with a crown-rust-resistant gene from Victoria, but also Southland which does not possess this gene. While these three isolates (No's. 27, 1, and 11) demonstrate pathogenicity as described for H. victoriae, the pathogenicity is quite different from that in the types used by Ivanoff (3) in that the isolates were pathogenic to Midsouth, as well as to Victorgrain 48-93.

Also included in this second group of isolates is isolate No. 15 (characteristic of only 4 of the isolates evaluated) which was able to rot some of the hosts, in addition to being able to blight them. It is believed that the ability to incite the rotting of the host represents an extreme degree of pathogenicity not exhibited by most of the isolates studied. The rotting of host tissues indicates a fungal invasion of the tissues, since it was possible to recover the fungus from these affected areas. Furthermore, the rapidity of host invasion (24 hours or less in our tests) represents a specialized pathogenic ability which characterizes isolates of this type.

The pathogenic ability of these Eu-helminthosporium isolates in terms of host response has been summarized in Table 2.

In Table 2 it is apparent that Groups I and IIa represent the pathogens capable of inciting culm rot and Victoria blight, respectively. However, it is also just as apparent that the isolates in Groups IIb and IIc may, or may not, be directly related to either or both of the first two groups. From the limited amount of data presented here, we cannot accurately determine the relationship among these four groups of isolates, but it is hoped that subsequent studies may provide the necessary information from which we can gain a better picture of these relationships.

Table 2. Grouping of *Eu-helminthosporium* isolates according to their ability to incite symptoms on four oat varieties.

Group	: Number of : isolates in : group	: Ability to incite		: Degree of : pathogenicity	: Representative : isolate
		: Culm : rot	: Victoria : blight		
I	3	+	0	High	No. 16
IIa	2	0	+	Low	No. 22
	23	0	+	Moderate	No. 27
IIb	6	+	+	Moderate	No. 1
	16	+	+	High	No. 11
IIc	4	+	+	High	No. 15 <sup>a</sup>

<sup>a</sup> Isolates of this group possess the ability to incite a rotting of host tissues within 24 hours, as well as possessing other pathogenicity.

The taxonomic separation of pathogenic organisms based upon host reactions to the attack by these pathogens is not a new concept in fungal classification, but one widely used by most uredinologists, and by many other mycologists. However, there has been some reluctance to use these same procedures to classify the Fungi Imperfecti. The bioassay described here, which gave critical host responses, was used to determine the presence and prevalence of certain *Eu-helminthosporium* species pathogenic upon oats in South Carolina. This same technique might have value in providing additional information for a more complete understanding of this group of fungi.

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EFFECT OF THE VIRUS YELLOWS ON SUGAR BEET SEED PRODUCTIONC. W. Bennett and J. S. McFarlane<sup>1</sup>Summary

Results of four field tests in 1956-57 and 1957-58 at Salinas, California, in which three selections of sugar beet were used, indicate that infection with yellows virus in March produced reductions in yields of seed ranging from 43.4 to 70.2 percent and that infection in May resulted in reductions ranging from 18.6 to 21.1 percent. There was a highly significant reduction in size of seeds of plants inoculated in March and a less significant reduction in size of seeds of plants inoculated in May. There was no evidence in these tests that yellows caused a reduction in viability of seeds.

## INTRODUCTION

Tests in Europe (1, 2) indicate that virus yellows of sugar beet may cause even greater losses to the seed crop than to the crop grown for sugar. Most of the sugar beet seed production in the United States is concentrated in the Salt River Valley of Arizona, western Oregon, southern Utah, and the Hemet Valley and the Tehachapi area of California. Yellows was first observed in the Salt River Valley in 1955, and high percentages of infection occurred in some fields in each succeeding year. Yields of seed were abnormally low in 1955, 1956, 1957, and 1958.

Yellows has been present in western Oregon at least since 1952. Information is not available on damage produced, but low yields in some fields in 1957 and 1958 indicate that the disease may become an important factor in seed production in that area. No evidence of serious losses caused by yellows in the Hemet Valley has been obtained. Yellows and beet mosaic, in combination, undoubtedly cause measurable reduction in yield of seeds in the Tehachapi area but the effects of yellows alone are difficult to determine.

The distribution of yellows indicates that it is likely to be a serious problem in seed production in all seed-producing areas in western United States. Experiments were initiated in 1956 in the Salinas Valley to obtain additional evidence on the effect of the disease on yield and quality of the seed crop.

## METHODS AND PROCEDURES

The mean temperatures prevailing in the Salinas Valley during the winter are too high for satisfactory seed production in commercial varieties of sugar beet. For this reason, it is necessary to use easy-bolting selections or to subject commercial varieties to periods of low temperature in cold-rooms before transplanting them to the field, to obtain satisfactory seed production.

An annual type of beet, SL 54484+0, obtained from F. V. Owen, was used in the 1956-57 test. The planting was made November 5, 1956. The plots were 12.5 feet long and four rows wide, and the plants were thinned to about 4 inches in the row. The treatments consisted of (a) inoculation with yellows virus March 18 before bolting started, (b) inoculation with yellows virus May 13 when the most advanced plants had a few open blossoms, and (c) no inoculation (check). Plots were inoculated also with curly top virus alone and in combination with yellows virus but no curly top infection was obtained. These plots were considered additional check and yellows plots, respectively. Results from these plots are shown in Table 1 along with results from the other plots in the test. Treatments were replicated four times. Plots were harvested August 8.

The variety US 33 and an easy-bolting inbred designated 6532-29 were used in tests in 1957-58. Plants of both types were started in the greenhouse in aluminum cylinders, stored 2 months in a room held at about 42° F for thermal induction, and then transplanted to the field in November. Treatments consisted of (a) inoculation with yellows virus March 4 when the plants were beginning to bolt, and (b) no inoculation (check). Plots consisted of 25 plants in each of four rows. Treatments were replicated six times.

An additional test was made with the inbred 6532-29 in field plots seeded directly Novem-

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Table 1. Results of tests at Salinas, California in 1956-57 and 1957-58 to determine the effects of virus yellows on yield and quality of sugar beet seed.

Variety or selection tested	Date inoculated with yellows virus	Calculated yield of seed per acre (pounds)	Calculated reduction in yield of seed per acre (percent)	Average weight of 100 seeds (grams)	Germination (percent)
SL 54484+0 (Direct seeding)	March 18, 1957	2068	44.5	1.421	53.9
	March 18, 1957 <sup>a</sup>	1981	43.4	1.434	52.5
	May 13, 1957	3034	18.6	1.680	62.6
	Uninoculated	3727	---	1.698	58.2
	Uninoculated <sup>b</sup>	3498	---	1.698	58.3
	L. S. D. at 5%	724	---	.250	NS <sup>c</sup>
	L. S. D. at 1%	1096	---	NS	NS
US 33 (Transplants)	March 4, 1958	2394	44.6	1.468	76.1
	Uninoculated	4321	---	1.732	79.1
	L. S. D. at 5%	535	---	.030	NS
	L. S. D. at 1%	838	---	.046	NS
6532-29 (Transplants)	March 4, 1958	1865	54.2	1.246	59.1
	Uninoculated	4072	---	1.520	57.1
	L. S. D. at 5%	599	---	.139	NS
	L. S. D. at 1%	939	---	.218	NS
6532-29 (Direct seeding)	March 4, 1958	793	70.2	1.245	59.4
	May 12, 1958	2098	21.1	1.358	51.1
	Uninoculated	2658	---	1.497	52.6
	L. S. D. at 5%	460	---	.150	NS
	L. S. D. at 1%	638	---	.208	NS

<sup>a</sup>Inoculated with both curly top and yellows viruses, but no curly top infection was observed.

<sup>b</sup>Inoculated with curly top virus, but no infection was observed.

<sup>c</sup>NS indicates that results are not significant.

ber 15, 1957. Treatments consisted of (a) inoculation with yellows virus March 4 before bolting started, (b) inoculation May 12 when fruiting stalks were about 12 inches tall, and (c) no inoculation (check). Treatments were replicated eight times. Plots were harvested August 5 and 6.

In both years, plots were sprayed at intervals from November until the plants were in blossom, to control aphids and reduce spread of yellows to check plots. A virulent strain of the yellows virus was used for inoculations in all tests.

## RESULTS

High percentages of plants in the check plots remained free of symptoms in the 1956-57 test and in the direct-seeded plots of the inbred 6532-29 in the 1957-58 test. However, there was some spread of virus to check plots in the transplants of the 1957-58 test as early as March and, by harvest time, more than 50 percent of the plants in the check plots showed evidence of infection. High percentages of infection were obtained in all inoculated plots.

The results of four tests are combined in Table 1, which shows the calculated yields of seed per acre, percent damage, average weight of 100 seeds, and percent germination of seed.

Yellows produced a marked reduction in yield of seeds in all tests. The inbred 6532-29 appears to have a relatively high degree of susceptibility to injury. Reduction in yield in March-inoculated beets reached 54.2 percent in the transplants and 70.2 percent in direct-seeded beets. Yellowing and necrosis were severe on mature leaves of plants, and the disease markedly reduced size of fruiting stalks and set of seed. Relative size of seedstalks and degrees of yellowing in check plots and plots of this inbred inoculated March 4 are illustrated in Figure 1.

Diseased plants produced smaller seeds in all tests. The reduction in seed size was significant at the 5-percent level in the March-inoculated plots of the 1956-57 test and at the



FIGURE 1. Seed beets of the inbred 6532-29 in an experimental planting in 1958, showing an uninoculated check plot (left) and plot inoculated March 4, 1958 with a virulent strain of the yellows virus (right). A row of the variety US 75 separates the two plots.

1-percent level in all other tests in which inoculations were made in March. May inoculations produced less reduction in seed size.

Germination of seeds was unusually low in all tests and there was a wide range of variation in germination of seeds from different plots, particularly in the 1956-57 test with the selection SL 54484+0. There is no evidence from these results that yellows caused a reduction in germination of seeds.

On the basis of the results obtained in these tests, it seems evident that if plants are infected with yellows before bolting begins, reduction in yield of seeds may be very high. Later infections apparently produce correspondingly lower reductions in yield. It seems evident, also, that there may be a wide range of variation among breeder's strains and perhaps among commercial varieties with respect to susceptibility to injury by the yellows disease.

#### Acknowledgment

The writers are indebted to Phyllis R. Emparan of the United States Agricultural Research Station, Salinas, California for assistance in harvesting plots and for making the seed-germination tests.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, SALINAS, CALIFORNIA

INHIBITION OF FUNGI BY ACROPHIALOPHORA NAINIANA<sup>1</sup>J. C. Edward and Richard D. Durbin<sup>2</sup>Abstract

*Acrophialophora nainiana* inhibits the growth of many fungi in agar. The optimum temperature for its growth is between 30° to 33° C and the optimum pH, 4.5 to 6.0. Thiamin and biotin increase the dry mat weight and antibiotic production of the fungus. Maximum antibiotic concentration is produced after 12 to 15 days and at pH 4.5 to 8.0. The antibiotic, equally effective at pH 4.5 to 8.0, is dialysable, thermostable, and neutral. Diethyl ether extracts at a concentration of 30 ppm completely inhibit the spore germination of *Helminthosporium sativum*.

While studying the microflora of field soil of the Allahabad Agricultural Institute, Allahabad, India, a fungus was isolated which strongly inhibited the growth of many fungi in soil dilution plates; the name *Acrophialophora nainiana* Edward<sup>3</sup> has been proposed for this fungus. Since in soil dilution plates the inhibitory effect of the fungus on the growth of a variety of fungi such as species of *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Pestalotia*, *Fusarium*, *Curvularia*, and *Mucor* was very striking, work was initiated on its antibiotic properties. Several fungi were grown separately with *A. nainiana* on malt agar medium at 30° C. *A. nainiana* is relatively slow-growing, and was inoculated into the medium 3 days before the test fungi. The test fungus was placed at a distance of 7 cm from the *A. nainiana* colonies and the zone of inhibition as a measure of antibiosis was recorded after a period of 2 weeks. (Table 1 and Fig. 1).

*A. nainiana* was grown on the six media listed for a period of 10 days and the cultural filtrates then tested for their effect on the germination of spores of *H. sativum*, *Fusarium oxysporum* f. *psidii*, and *Curvularia lunata*.

1. Czapek's medium
2. Czapek's with glucose replacing sucrose
3. Czapek's plus 0.5% yeast extract
4. Czapek's plus 0.2% asparagine
5. Czapek's plus inositol (5 mg/liter), thiamin (100 µg/liter) and biotin (5 µg/liter).
6. Potato broth plus 2% glucose

The results of this test indicated that the effect of the filtrate from any single medium was similar on all three test fungi and that the last four media were equally effective and better than the first two.

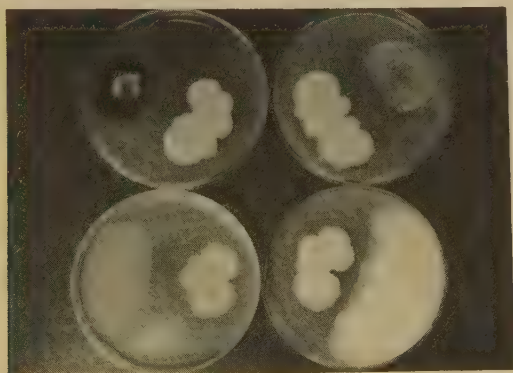


FIGURE 1. Growth inhibition of four fungi by *A. nainiana*. The test fungi in the top left and right plates are *Helminthosporium sativum* and *Monosporium apiospermum* and in the lower left and right plates *Fusarium oxysporum* f. *psidii* and *Polyporus versicolor*.

<sup>1</sup> Paper No. 4216, Scientific Journal Series, Minnesota Agricultural Experiment Station.

<sup>2</sup> Research Fellow and Assistant Professor, respectively, Department of Plant Pathology and Botany, University of Minnesota. This work was done while the senior author was on a Rockefeller Foundation Post-doctoral Fellowship.

The authors wish to thank Professor J. J. Christensen, Head of the Department of Plant Pathology and Botany, for his encouragement during the investigation, and Mr. B. P. Chakravarti for his help in photography.

<sup>3</sup> Edward, J. C. 1959. A new genus of Moniliaceae. *Mycologia* (in press).

Table 1. Inhibition of growth of seven test fungi by *A. nainiana*.

Organism	Zone of inhibition (mm) <sup>a</sup>
<i>Aspergillus niger</i> Tiegh.	12
<i>Curvularia lunata</i> (Walker) Boedijn	18
<i>Fusarium oxysporum</i> f. <i>psidii</i> Prasad, Mehta, & Lal.	18
<i>Helminthosporium sativum</i> Pam., King & Bakke	20
<i>Monosporium apiospermum</i> Sacc.	15
<i>Penicillium</i> sp.	10
<i>Polyporus versicolor</i> (L.) Fr.	10

<sup>a</sup> Each figure is the mean of three replications.Table 2. The effect of temperature on mat weight and antibiotic production by *A. nainiana*.

Temperature	Mean mat weight (gm) <sup>a</sup>	Germination inhibition of <i>Helminthosporium sativum</i> spores
25	0.166	partial
30	0.370	complete
33	0.351	complete
39	0.282	partial
43-45	0.001	none

<sup>a</sup> Mean weight of three replications.Table 3. The effect of pH on mat weight and antibiotic production by *A. nainiana*.

Initial pH	Final pH	Mean mat weight (gm) <sup>a</sup>	Germination inhibition of <i>Helminthosporium sativum</i> spores
3.0	3.5	0.155	--
3.5	6.0	0.233	partial
4.5	6.8	0.376	complete
6.0	6.8	0.313	complete
7.0	7.0	0.150	complete
8.0	8.0	0.146	complete

<sup>a</sup> Mean weight of three replications.Table 4. The inhibitory effect of the cultural filtrate of *A. nainiana* on the germination of spores of 12 fungi and the vegetative growth of 2 bacteria and 1 alga.

Test organisms	Inhibition <sup>a</sup>
<i>Alternaria tenuis</i> Nees	partial
<i>Aspergillus niger</i> Tiegh.	complete
<i>Curvularia lunata</i> (Walker) Boedijn	complete
<i>Erysiphe graminis</i> DC.	complete
<i>Fusarium oxysporum</i> f. <i>psidii</i> Prasad, Mehta & Lal.	complete
<i>Helminthosporium sativum</i> Pam., King & Bakke	complete
<i>Monosporium apiospermum</i> Sacc.	complete
<i>Penicillium</i> sp.	complete
<i>Puccinia graminis</i> Pers. f. <i>sp. tritici</i> Eriks. & E. Henn.	complete
<i>Saccharomyces ellipsoideus</i> Hansen	partial
<i>Trichoderma viride</i> Pers. ex Fr.	complete
<i>Ustilago hordei</i> (Pers.) Lagh. (Fisher, 1953)	complete
<i>Escherichia coli</i> (Migula) Castellani & Chalmers	none
<i>Staphylococcus aureus</i> Rosenbach	none
<i>Chlorella ellipsoidea</i> Gerneck	none

<sup>a</sup> The observation recorded is based on tests replicated twice.

Spores of *H. sativum* were used for bioassay of the antibiotic since, at different dilutions, the antibiotic had a characteristic effect on spore germination. The undiluted cultural filtrate completely inhibited germination; at dilutions of 1:1 and 1:2 it induced production of vesicular cells and witches' broom-like hyphae, and at a dilution of 1:3 beaded hyphae were produced (Figs. 2A-C).

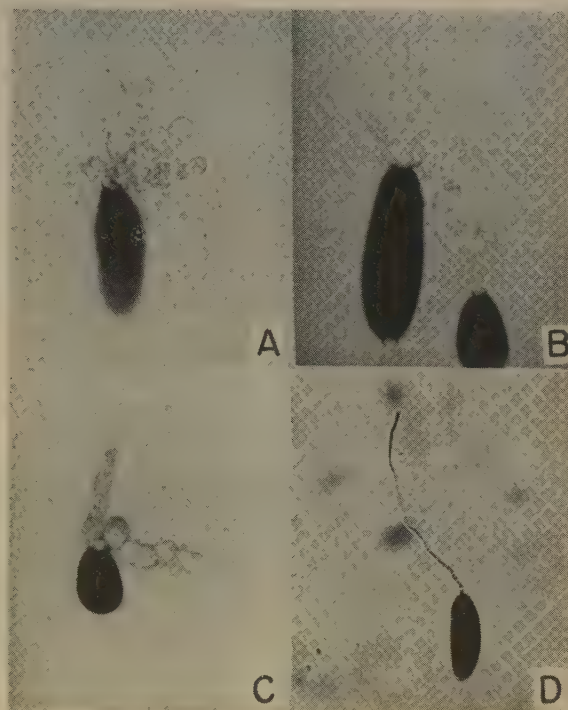


FIGURE 2. Production of:  
A -- vesicular cells at 1:1 dilution.  
B -- Witches' broom-like germ tubes at 1:2 dilution. C -- beaded germ tubes at 1:3 dilution. D -- normal germ tubes in a water control.

In experiments where dry mat weights of the fungus were determined, 3-mm discs of 7-day-old cultures of the fungus on malt agar medium served as inoculum. The fungus mats were harvested after 2 weeks' growth at 30° C, dried at 90° for 48 hours, and the dry weights determined. Each treatment was replicated three times and the mean calculated.

Thiamin, biotin, and inositol, either singly or in all possible combinations, were added to Czapek's medium to study their influence on the production of the antibiotic. It was found that both thiamin and biotin were necessary for maximum antibiosis. Thereafter, all studies on antibiotic production were conducted using Czapek's medium supplemented with thiamin (100 µg/liter) and biotin (5 µg/liter).

The fungus was grown on Czapek's supplemented with thiamin and biotin and on Czapek's alone to measure the influence of thiamin and biotin on dry weight of mats. The mean dry mat weight from the medium with and without the vitamins was 0.345 g and 0.271 g, respectively, and, as would be expected, the filtrate from the medium supplemented with vitamins was more inhibitory to spore germination.

Optimum temperature of five tested for dry mat weight was between 30° and 33° C (Table 2). A direct correlation was observed between mat weight and antibiosis.

A time study on antibiotic production by the fungus grown at 30° C in the usual medium indicated the following facts. The production of antibiotic was observable from the fourth day on, increased to 11th to 12th day, after which it was steady until 14th to 15th day, then tended to diminish.

The pH of the cultural filtrate was adjusted to 3, 3.5, 4.5, 6, 7, and 8 and then tested for inhibitory effect on spore germination. A parallel germination test with water adjusted to the same pH's served as a control. At pH 3 germination was inhibited even in the water control, and inhibition due to the antibiotic was complete between pH 4.5 and 8, with partial inhibition at pH 3.5.

The influence of the pH of the medium on mat weight and antibiotic production was determined. The pH adjustments for pH's below 6 were made with citric acid (0.1M) and above 6 with dipotassium hydrogen phosphate (0.2M). Mat weight increased with an increase in pH to

4.5 and thereafter dropped (Table 3). Little antibiotic appeared to be produced at pH's 3.0 and 3.5, whereas from pH 4.5 to 8.0 the antibiotic effect was about the same, all filtrates completely inhibiting spore germination.

The culture filtrate was tested for its inhibitory effect on germination of spores of 12 different fungi and the vegetative growth of two bacteria and one alga. Complete inhibition of spore germination was observed in all fungi except Alternaria tenuis and Saccharomyces ellipsoideus, where the inhibition was only partial. The alga (Chlorella ellipsoidea Gerneck) and the bacteria (Escherichia coli (Migula) Castellani and Chalmers, Staphylococcus aureus Rosenbach), however, were unaffected (Table 4).

Several organic solvents were tested for their ability to extract the inhibitory principle from the aqueous cultural filtrate. This was done by thoroughly shaking the cultural filtrate with one-third of its volume with the solvent in a separatory funnel. Generally, each moiety of the filtrate was extracted three times to insure maximum extraction. The organic solvent extract was allowed to evaporate under a heat lamp, the residue dissolved in a known volume of water, and then tested for its inhibitory effect on spore germination. Extracts made with petroleum ether and carbon tetrachloride had no inhibitory effect, whereas that made with benzene had some. On the other hand, diethyl ether and n-butanol extracts were found to be highly inhibitory; diethyl ether extract inhibited even the growth of the alga and bacteria, and at a concentration of 30 ppm completely inhibited germination of H. sativum spores.

The antibiotic principle was found to be dialysable, neutral, and stable when subjected to heat at 100° C for 15 minutes.

The antibiotic produced by the fungus has a wide antifungal spectrum. Work on methods of augmenting antibiotic production, of extraction, and of purification is now in progress.

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# A MOIST-CHAMBER TECHNIQUE FOR CULTURING LARGE NUMBERS OF ELM TWIG SPECIMENS

Abraham H. Epstein

With the discovery of the Dutch elm disease in Wisconsin in 1956, the State Department of Agriculture embarked on an intensive program of survey and control. Specimens from suspect elm trees are cultured without charge in the laboratory maintained by the Division of Plant Industry.

In 1958, when the disease was reported from only 10 out of a total of 71 Wisconsin counties, 3782 specimens were submitted to the laboratory for culturing. It was anticipated that there would be a large increase in the number of specimens submitted as the disease progressed into other counties. This would most certainly tax the laboratory, which was already operating at peak capacity during part of the season.

During the winter of 1958-59, the writer recovered *Ceratocystis ulmi* (Buis.) Moreau from dead trees on a number of occasions using moist chambers. It was then decided to attempt to adapt the moist-chamber technique to culture of fresh specimens on a mass scale. The main object was to reduce the time required for culture preparations as well as space for storage during incubation. A number of containers and various media were tried.

To date the best results have been obtained using wide mouth square flint glass bottles (Cenco #10405) of 2-ounce capacity with metal screw caps as the moist chambers, and a 3 percent solution of sucrose fortified with 1 percent yeast extract as the medium. A quantity of this medium sufficient to cover the bottom of the bottle to a depth of 1/8 inch (about 2 1/3 cc's) is pumped into the bottle by means of a plastic wash bottle (Fig. 1). The bottles are then loosely capped and autoclaved at 15 pounds of pressure for 20 minutes.

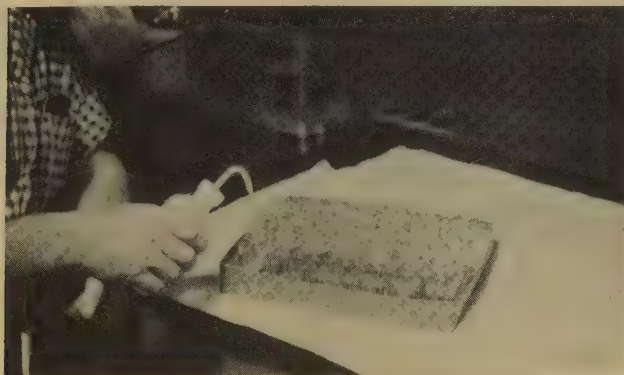


FIGURE 1. Injecting nutrient solution into culture bottles prior to sterilization.



FIGURE 2. Placing sections of specimen twigs in culture. (Wide mouth bottle contains alcohol for flaming the pruning shears.)

The procedure for placing the specimen in culture is as follows: Groups of 10 autoclaved bottles and media are placed in a special rack which holds them at an angle of about 20° with the cap end out and facing the technician. The bark is then peeled lengthwise from a specimen twig taking care to prevent contamination of the exposed wood. Utilizing a pair of hand pruning shears which have been dipped in alcohol and flamed, a 1/2-inch section is cut from the distal end of the peeled specimen and discarded to eliminate organisms contaminating the cut ends of the specimen twigs during transit from the field to the laboratory. The cap of the prepared culture bottle is removed and three or four sections, 1/2 inch in length are cut directly into the bottle and the cap is quickly replaced (Fig. 2). Laboratory specimen numbers are applied with a glass marking pen and the bottles are then placed in racks for incubation.

Coremia are produced on the wood and also on a pellicle of mycelium which forms on the surface of the media. The average incubation period is 5 to 6 days, which is comparable to the potato dextrose agar-Petri dish method. By using mycelial characteristics only, the culture may be read in 2 to 3 days. A binocular is used for examining the cultures at 18 and 36 magnification.

With this technique, one technician is able to process approximately 40 specimens per hour as compared with about 20 per hour with the agar-plate method. There is also a definite saving of time in preparation of media and in cleaning of culture containers as well as reduced cost of media.

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SUSCEPTIBILITY OF TOMATO  
(LYCOPERSICON ESCULENTUM) TO THE SUGAR-BEET NEMATODE  
(HETERODERA SCHACHTII)<sup>1</sup>

A. Morgan Golden and Thelma Shafer<sup>2</sup>

Practical control of the sugar-beet nematode, Heterodera schachtii Schmidt, is based primarily on a system of crop rotation whereby only non-host crops are grown on nematode-infested fields for about 4 years. This practice permits natural decline of the nematode population so that a crop of sugar beets (Beta vulgaris) can then be successfully grown with little or no visible injury. Tomato (Lycopersicon esculentum), an economically important crop in many beet-producing areas of the United States, has long been recommended (5, 6) and used as a non-host crop in the rotation system for the control of the sugar-beet nematode.

Despite the established use of tomato in rotations with sugar beets and the fact that both Jones (2) and Mulvey (3) did not observe nematode cysts on the roots of this plant growing in small, infested field plots, tomato was recently found to be susceptible to the sugar-beet nematode in tests at Salinas, California. Twenty-five young tomato plants (Pearson XL, a red variety) and the same number of sugar-beet seedlings were grown in the greenhouse for 54 days in individual aluminum-foil cylinders containing soil heavily infested with cysts of H. schachtii. This procedure, as well as the method of examination of the plants and of rating the infection, was essentially the same as described by Golden and Shafer (1). Twenty-two of the tomato plants had an infection-index rating of 2.0 (light), two had a rating of 3.0 (moderate), and one had a rating of 4.0 (heavy), giving an average infection-index rating of 2.1. All the sugar beets were heavily infected with the sugar-beet nematode, having an average infection-index rating of 4.0. Microscopic examination of gravid white females and young cysts from the tomato roots revealed the presence of viable eggs and larvae. These specimens appeared to be morphologically similar to H. schachtii. When gravid females and young cysts from tomato were placed on potted sugar beets, a population of nematodes similar to H. schachtii was established.

Although no specimens were seen during brief visual examination of tomato roots in the field, several gravid white females and obviously young cysts similar to H. schachtii were recovered by washing and sieving the soil and roots of tomato growing in two different fields known to be infested with sugar-beet nematode. These specimens presumably developed on the roots of tomato, as the fields were almost free of other plants. In another nearby infested field in which infected sugar beets were growing, a few white gravid females were found attached to the roots of Solanum sarrachoides. These nematodes also appeared to be similar to H. schachtii, but definite identification was not made because cysts and sufficient second-stage larvae were lacking.

In host-range studies of the sugar-beet nematode, Raski (4) found a few females and cysts on two yellow varieties of tomato (Golden Queen and Jubilee) but none on a red variety (Earliana). Sugar beet was infected by inoculation with females and cysts which developed on the Golden Queen tomato. The failure of Raski (4) to find females and cysts on the Earliana variety suggests the possibility of resistance to H. schachtii within the widely used red varieties, a desirable characteristic to have in tomato for growth in the beet-producing areas.

Further tests are being conducted on the susceptibility to the sugar-beet nematode of several tomato varieties and on the effect of tomato on the nematode population. In view of the results so far, and because of the long persistence of H. schachtii cysts in the soil, the use of susceptible tomato varieties or other host plants in a rotation system designed to reduce the population of sugar-beet nematode should be avoided.

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<sup>1</sup> Cooperative Investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and The Beet Sugar Development Foundation.

<sup>2</sup> Respectively, Nematologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Salinas, California, and Assistant Nematologist, Beet Sugar Development Foundation, Salinas, California.

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*Puccinia coronata*

LANDHAFER RACES OF CROWN RUST ON OATS  
IN SOUTH TEXAS AND NORTHERN MEXICO IN 1959<sup>1</sup>

M. C. Futrell, Manuel Cáñero and Lucas Reyes<sup>2</sup>

Summary

Landhafer attacking races of crown rust of oats were present in south Texas and northern Mexico in epiphytotic proportions for the first time during the 1958-59 season. Spore showers occurred early in the season; temperature, humidity and other environmental conditions were ideal for establishing crown rust. This disease did not spread northward across Texas because of barriers in environmental factors.

INTRODUCTION

Prior to the growing season of 1958-59, the Landhafer attacking races of crown rust had been found in only trace amounts in Texas and northern Mexico. These races of rust had been known to be present in the southeastern United States for the past 5 years.

EXPERIMENTAL PROCEDURE AND RESULTS

Three varieties of oats, Minhafer, Suregrain and Floriland, were seeded in drill strips at Rio Bravo, Tamaulipas, and Monterrey, Nuevo Leon, Mexico<sup>3</sup>, and at Beeville, Prairie View, College Station, Denton and Iowa Park, Texas to determine whether Landhafer attacking races of crown rust were present in these areas. The parentage of these varieties are as follows: Minhafer -- (Bond-Rainbow x Hajira-Joanette) x Landhafer; Floriland -- Florida 167 x Landhafer; and Suregrain -- Arlington-Delair x Trispermia. Minhafer and Floriland derived their resistance from Landhafer and are susceptible to both the race 264 group and the 290 group. Suregrain derived its resistance from Trispermia and is resistant to the 290 group but susceptible to the 264 group of crown rust races. Vaseline slides were exposed at Rio Bravo, Mexico from late December through March 20, 1959 to determine when spore showers occurred in that area.

The oats in plots emerged at Rio Bravo on December 27, 1958 and a number of spore showers followed. Environmental factors were ideal for rust development. Showers of leaf rust spores occurred from December 28 to January 3. A second shower occurred on January 8 and 9, and a third shower was observed from January 19 through 21 (Table 1). Rain, drizzle, fog, and cloudy weather afforded ideal weather for the development of rust. On January 19 and 20, the maximum temperature was 78° F and the lows were 66° and 58°, which are ideal for rust development. As can be seen from Table 1, temperatures and other environmental factors remained favorable for rust development at Rio Bravo from January through March.

Crown rust developed during the winter on Minhafer and Floriland, but Suregrain remained free from rust at Monterrey, Rio Bravo and Beeville. Lower temperatures prevented the development of crown rust on any of the varieties at Prairie View, College Station and other stations in north Texas. Reading of rust was taken at each station in early April and the responses of varieties by stations are given in Table 2.

The data show that conditions were ideal for the establishing of crown rust in northern Mexico and south Texas during the winter of 1958-59. Northward at College Station and Prairie View, environmental conditions were far less favorable for rust establishment. Crown rust failed to survive the winter north of College Station.

<sup>1</sup> Texas Agricultural Experiment Station, Technical Article No. 3304. Cooperative investigations conducted by the Texas Agricultural Experiment Station, Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Estacion Agricola Experimental, Instituto de Investigaciones Agricolas, Secretaria de Agricultura y Ganaderia, Rio Bravo, Tamaulipas, Mexico.

<sup>2</sup> Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, College Station, Texas; Superintendent Estacion Agricola Experimental, Instituto de Investigaciones Agricolas, Secretaria de Agricultura y Ganaderia, Rio Bravo, Tamaulipas, Mexico; and Jr. Agronomist, Texas Agricultural Experiment Station, Beeville, Texas.

<sup>3</sup> The cooperation of Leonel Robles and Martin Cruz for growing plots of Minhafer and Suregrain at Apodaca (Monterrey, Nuevo Leon, Mexico), is acknowledged.

Table 1. Daily record of temperature, weather, wind direction and showers of crown rust spores at Rio Bravo, Tamaulipas, Mexico.

Date	Temperature (°F.)		Weather	Wind direction	Urediospore count	
	Max.	Min.			L R <sup>a</sup>	S R <sup>a</sup>
1958						
12-27	64	52	Cloudy	N	0	0
12-28	68	58	P. <sup>b</sup> cloudy	N	0	0
12-29	66	46	P. cloudy	N	144	0
12-30	42	37	Drizzle	N	480	0
12-31	40	33	P. cloudy	N-NW	48	0
1959						
1- 1	44	40	Rain	SE	96	96
1- 2	70	41	P. cloudy	N	0	144
1- 3	68	33	P. cloudy	SE	288	40
1- 4	36	32	Cloudy	N	0	96
1- 5	40	31	Rain	N	0	48
1- 6	40	34	Rain	N	0	48
1- 7	56	42	Drizzle	N	0	0
1- 8	58	44	Fair	N	144	96
1- 9	52	31	Fair	N	48	0
1-10	61	40	Fair	N	0	0
1-11	68	54	Drizzle	N-SE	0	0
1-12	68	56	Drizzle	SE	0	96
1-13	68	58	P. cloudy	SE	0	0
1-14	70	58	Fair	SE	48	0
1-15	68	40	Clear	SE-N	0	0
1-16	58	37	Clear	N	0	0
1-17	64	46	P. cloudy	N-SE	0	0
1-18	65	48	Cloudy	SE	0	0
1-19	78	66	P. cloudy	SE	720	48
1-20	78	58	P. cloudy	SE-N	336	48
1-21	60	33	Clear	N	48	0
1-22	60	40	Clear	N	0	0
1-23	68	50	Drizzle	NE	0	0
1-24	64	59	Drizzle	NE	0	0
1-25	73	55	P. cloudy	NE	0	0
1-26	77	54	Clear	NE	0	0
1-27	72	54	Cloudy		Broken slide	
1-28	51	46	Cloudy	N	0	0
1-29	68	50	Cloudy	N	96	0
1-30	61	55	Rain	N	144	0
1-31	54	49	Rain	N	0	0
2- 1	84	64	P. cloudy	NE	0	0
2- 2	41	36	Rain	N	0	0
2- 3	37	34	Rain	N	48	48
2- 4	72	39	Clear	NE	144	0
2- 5	73	40	Clear	NE	0	0
2- 6	61	48	Clear	NE	0	0
2- 8	72	66	P. cloudy	SE	912	0
2-10	72	61	Rain		Damaged slide	
2-11	50	46	Rain	N	96	0
2-12	51	43	Rain		Damaged slide	
2-13	77	68	P. cloudy	NE	1200	0
2-14	75	68	Rain	N	1104	0
2-15	64	57	Rain		Damaged slide	
2-16	72	43	P. cloudy	S	192	0
2-17	88	52	Clear	SE	624	336
2-18	88	59	Clear	E-N	912	288
2-19	71	53	Rain	N	576	240
2-20	70	54	Rain		Damaged slide	
2-21	55	46	Rain	N	0	0
2-22	72	54	Cloudy	N-SE	144	0
2-23	72	61	Rain	S-N	96	0
2-24	73	55	Rain		Damaged slide	
2-25	61	56	Rain	N	0	0
2-26	65	54	Drizzle	N	96	48
2-27	70	50	Cloudy	N	48	0
2-28	75	47	Clear	N	384	0
3- 1	80	49	Clear	N-SE	576	144
3-2,3	80	53	P. cloudy	SE-N	528	0
3- 4	80	52	P. cloudy	N-SE-N	3360	0
3- 5	68	37	P. cloudy	N	96	0
3- 6	68	47	P. cloudy	N-SE	0	0
3- 7	67	42	Rain	SE	0	0
3- 8	75	55	Rain		Damaged slide	
3- 9	81	64	P. cloudy	SE	96	0
3-10	81	70	P. cloudy	SE	960	0
3-11	84	53	P. cloudy	SE-N	3388	48
3-14	90	49	P. cloudy	SE-N	336	0
3-16	69	48	Clear	N	528	0
3-17	53	43	Rain		Damaged slide	
3-18	64	45	P. cloudy	N-SE	240	48
3-19	77	52	P. cloudy	SE	864	0
3-20	86	54	P. cloudy	SE-N	1248	0

<sup>a</sup> L R = Leaf rust; S R = Stem rust<sup>b</sup> Partly cloudy

Table 2. Response of three oat varieties to crown rust at five stations on April 1, 1959.

Variety	Monterrey	Rio Bravo	Beeville	Prairie View	College Station
Suregrain	0	0	0	0	0
Minhafer	30 S <sup>a</sup>	30 S	10 S	1 S	1 S
Floriland	-- <sup>b</sup>	30 S	10 S	1 S	1 S

<sup>a</sup> Figures = percent; S = susceptible.

<sup>b</sup> Not grown at this station.

The northward spread of crown rust in the spring of 1959 was very slow; and due to cool, dry weather the Landhafer attacking races never became established in indicator plots of Minhafer and Floriland at Denton and Iowa Park.

A large acreage of oats is grown in south Texas for pasture, and data indicate that a small amount of inoculum of a race of rust can be very effective in establishing an epiphytotic when a susceptible host is present.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, AND THE TEXAS AGRICULTURAL EXPERIMENT STATION, COLLEGE STATION, TEXAS; SUPERINTENDENT ESTACION AGRICOLA EXPERIMENTAL, INSTITUTO DE INVESTIGACIONES AGRICOLAS, SECRETARIA DE AGRICULTURA Y GANADERIA, RIO BRAVO, TAMAULIPAS, MEXICO

PRELIMINARY SURVEY OF PLANT DISEASES IN THE REPUBLIC OF PANAMA, 1955-1958R. W. Toler, Rogelio Cuellar, and Juan B. Ferrer<sup>1</sup>

Prior to the survey herein reported, scattered references to plant diseases in the Canal Zone and two definitive records were published. Cralley<sup>2</sup> noted the occurrence and economic importance of certain plant diseases in the Republic of Panama following a survey made in 1952, and Lucas<sup>3</sup> reported on tobacco diseases in the Chiriqui Province of Panama in 1958.

This survey was undertaken to determine the importance of disease losses to Panamanian agriculture to serve as a guide for pathological research, to detect diseases that could be of potential danger to crops in the Western Hemisphere and the United States, and to evaluate the feasibility of establishing a plant quarantine service in the country. The last two objectives were prompted by Panama's strategic position in the Western Hemisphere as a crossroad for world shipping. For example, the rice stripe virus known as "hoja blanca" was first observed in Panama and the Western Hemisphere by Cralley in 1952<sup>2</sup>. Further, there has been an increase in the production of fresh market crops in Panama for marketing in the Canal Zone and for the winter market in the United States.

The plant diseases found occurring on the more important economic crops are grouped according to host. The common name of the diseases are given in English and Spanish, along with brief remarks on the relative economic importance of the diseases in Panama (Table 1).

## DISCUSSION

Although this list of plant diseases will be expanded on the crops listed, the survey extended to additional crops, and new diseases introduced, this report includes the more important destructive diseases in the country at present.

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<sup>2</sup>Cralley, E. M. 1954. Plant diseases observed in Panama in 1952. (Enfermedades de las Plantas en Panama. Ministerio de Agricultura, Comercio e Industrias y Servicio Interamericano de Cooperacion Agricola en Panama, R. de Panama 1954).

<sup>3</sup>Lucas, G. B. 1958. Tobacco diseases in Panama. Plant Disease Reprtr. 42:1301.

Table 1. Plant diseases observed in 1955-1958 survey in the Republic of Panama.

Host and pathogen	Common name of disease (English, Spanish)	Relative economic importance	Host and pathogen	Common name of disease (English, Spanish)	Relative economic importance
<b>BANANA</b> ( <i>Musa paradisica</i> and <i>M. sapientum</i> )			<b>Table 1. (continued)</b> <b>CITRUS, ORANGE, GRAPEFRUIT AND TANGERINE</b> ( <i>Citrus</i> spp.) (Continued)		
<i>Diplodia theobromae</i>	Fruit rot	Minor importance	<i>Trichoderma</i> spp.	Needle nematodes	Economic losses undetermined
<i>Cercospora musae</i>	Pudrición del guineo	Widespread and damaging	<i>Xiphinema</i> spp.	Nematodes	Economic losses undetermined
<i>Fusarium cubense</i>	Sigatoka	Limiting produc- tion	<b>COCONUT PALM</b> ( <i>Cocos nucifera</i> )		
<i>Sclerotium rolfsii</i>	Mal de Panama	Losses compara- tively light	<i>Phytophthora palmivora</i>	Bud rot	Severe in local- ized areas
<i>Pseudomonas solanacearum</i>	Pudrición de la raíz	Potential danger	<i>Pestalotia palmarum</i>	Gray leaf spot	Widespread, minor importance
<i>Meloidogyne</i> sp.	Bacterial wilt	Economic loss undetermined	<i>Aphelenchoides cocophilus</i>	Mancha gris	Minor importance
	Moko			Red ring	
	Root-knot			Anillo rojo	
	Hernias radicales		<b>COFFEE</b> ( <i>Coffea arabica</i> )		
<b>BEAN</b> ( <i>Phaseolus vulgaris</i> )			<i>Capnodium</i> spp.	Sooty mold	Widespread, no economic importance
<i>Pythium</i> spp.	Cottony leak	Heavy losses in transit	<i>Corticium salmonicolor</i>	Fumagina	Severe in local- ized areas
<i>Sclerotinia sclerotiorum</i>	Pudrición derania	Widespread and damaging	<i>Pellicularia koleroga</i>	Dieback	Severe in local- ized areas
<i>Colletotrichum lindemuthianum</i>	White mold	Minor importance	<i>Mycena citricolor</i> <sup>a</sup>	Thread blight	Widespread losses
<i>Rhizoctonia</i> sp.	Espumilla	Heavy losses in the field	<i>Cercospora coffeicola</i>	Mal de hilacha	Damage slight
<i>Sclerotium rolfsii</i>	Anthraxnose	Severe in local- ized areas	<i>Colletotrichum coffeamum</i> <sup>a</sup>	Leaf spot	
	Anthracnose		<i>Fusarium</i> spp., <i>Rhizoctonia</i> spp., and <i>Phytophthora</i> spp.	Ojo de gallo	
	Web blight		<i>Phyllosticta</i> sp.	Leaf spot	
	Pudrición de la hoja		<i>Meloidogyne</i> spp.	Mancha foliar	
	Southern blight		<i>Pratylenchus</i> spp.	Root-knot	
	Pudrición del tallo		<i>Helicotylenchus</i> sp.	Hernias radicales	
<b>CABBAGE</b> ( <i>Brassica oleracea</i> )			<i>Trichoderma</i> spp.	Lesión nematodes	
<i>Peronospora parasitica</i>	Downy mildew	Minor importance	<i>Xiphinema</i> spp.	Nematodes	
<i>Phoma lingam</i>	Mildiu	Minor importance		Nematodes	
<i>Xanthomonas campestris</i>	Leaf spot	Severe in local- ized areas		Nematodes	
<i>Erwinia carotovora</i>	Mancha de la hoja	Losses compara- tively light		Nematodes	
	Black rot			Nematodes	
	Podredumbre negra			Nematodes	
	Soft rot			Nematodes	
	Podredumbre suave			Nematodes	
<b>CACAO</b> ( <i>Theobroma cacao</i> )				Nematodes	
<i>Phytophthora palmivora</i>	Black rot	Losses compara- tively light		Nematodes	
<i>Monilia roleri</i>	Pudrición negra	Widespread and limiting		Nematodes	
	Pod rot			Nematodes	
	Monilliasis			Nematodes	
<b>CANTALOUPE</b> ( <i>Cucumis melo</i> )				Nematodes	
<i>Pseudoperonospora cubensis</i> <sup>a</sup>	Downy mildew	Widespread and damaging		Nematodes	
<i>Erysiphe cichoracearum</i>	Mildiu	Widespread and severe		Nematodes	
<i>Cladosporium cucumerinum</i>	Powdery mildew	Severe in local- ized areas		Nematodes	
<i>Colletotrichum lagenarium</i>	Cenicilla	Minor importance		Nematodes	
<i>Sclerotium rolfsii</i>	Scab	Severe in local- ized areas		Nematodes	
<i>Meloidogyne</i> sp. <sup>a</sup>	Roña	Severe in local- ized areas		Nematodes	
	Anthraxnose			Nematodes	
	Anthracnose			Nematodes	
	Southern blight			Nematodes	
	Pudrición del cuello			Nematodes	
	Root-knot			Nematodes	
	Nudo de la raíz			Nematodes	
<b>CARROT</b> ( <i>Daucus carota</i> var. <i>sativa</i> )				Nematodes	
<i>Cercospora carotae</i>	Leaf spot	Yields not affected		Nematodes	
<i>Erwinia carotovora</i>	Mancha de la hoja	Widespread, damage moderate		Nematodes	
	Soft rot			Nematodes	
	Podredumbre suave			Nematodes	
<b>CASSAVA</b> ( <i>Manihot esculenta</i> )				Nematodes	
<i>Phytophthora</i> sp.	Root rot	Reduction in stands		Nematodes	
<i>Botrytis</i> sp.	Pudrición de la raíz	Severe in local- ized areas		Nematodes	
<i>Cercospora caribaea</i>	Bud rot	Minor importance		Nematodes	
<i>Gloeosporium</i> sp.	Terminal muerto	Losses undeterm- ined		Nematodes	
<i>Rhizoctonia solani</i>	Leaf spot	Losses compara- tively light		Nematodes	
	Manchablanca			Nematodes	
	Dieback			Nematodes	
	Babilla			Nematodes	
	Root rot			Nematodes	
	Pudrición de la raíz			Nematodes	
<b>CHAYOTE</b> ( <i>Sesquium edule</i> )				Nematodes	
<i>Cercospora sechii</i>	Leaf spot	Damage only slight		Nematodes	
<i>Cladosporium cucumerinum</i>	Mancha foliar	Severe in local- ized areas		Nematodes	
<i>Colletotrichum lagenarium</i>	Scab, foliage and fruit	Losses only mod- erate		Nematodes	
	Roña			Nematodes	
	Anthracnose			Nematodes	
	Anthracnose			Nematodes	
<b>CITRUS, ORANGE, GRAPEFRUIT AND TANGERINE</b> ( <i>Citrus</i> spp.)				Nematodes	
<i>Phytophthora parasitica</i> <sup>a</sup>	Brown rot, gummosis	Severe and wide- spread		Nematodes	
<i>Capnodium citri</i> <sup>a</sup>	Gomosis	Widespread		Nematodes	
<i>Diaporthe citri</i> <sup>a</sup>	Sooty mold	Widespread and limiting production		Nematodes	
<i>Corticium salmonicolor</i>	Fumagina	Widespread		Nematodes	
<i>Aspergillus</i> spp. and <i>Penicillium</i> spp.	Melanose	Severe		Nematodes	
<i>Cladosporium citri</i>	Melanosis	Minor importance		Nematodes	
<i>Colletotrichum gloeosporioides</i>	Pink disease	Minor importance		Nematodes	
<i>Oidium tingtonianum</i>	Mal rosado	Severe in local- ized areas, poten- tial danger		Nematodes	
<i>Sphaceloma fawcettii</i> <sup>a</sup>	Fruit rot	Widespread and damaging		Nematodes	
<i>Rhizoctonia solani</i>	Pudrición de la fruta	Severe on seedlings		Nematodes	
<i>Virus</i> <sup>a</sup>	Leaf mold	Losses light, potential danger		Nematodes	
<i>Tylenchorhynchus</i> sp.	Moho negro	Economic losses undetermined		Nematodes	
<i>Helicotylenchus</i> sp.	Anthraxnose	Economic losses undetermined		Nematodes	
	Powdery mildew			Nematodes	
	Mildiu			Nematodes	
	Scab			Nematodes	
	Roña			Nematodes	
	Damping-off			Nematodes	
	Mal del talluelo			Nematodes	
	Quick decline virus			Nematodes	
	Tristeza			Nematodes	
	Stunt nematodes			Nematodes	
	Nematodes			Nematodes	
	Spiral nematodes			Nematodes	
	Nematodes			Nematodes	

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Table 1. (continued)

Host and pathogen	Common name of disease (English, Spanish)	Relative economic importance
<b>EGGPLANT</b> ( <i>Solanum melongena</i> )		
<i>Phomopsis vexans</i>	Fruit rot Podricion de la fruta	Losses light
<i>Rhizoctonia solani</i>	Damping-off Mal de almácigo	Losses comparatively light
<i>Sclerotium rolfsii</i>	Southern blight Podricion del cuello	Severe in localized areas
<i>Pseudomonas solanacearum</i>	Bacterial wilt Marchitez bacteriana	Severe and limiting
<i>Meloidogyne incognita</i>	Root-knot Hernias radicales	Severe in localized areas
<b>ONION</b> ( <i>Allium cepa</i> )		
<i>Peronospora destructor</i>	Downy mildew Mildiu velloso	Minor importance
<i>Aspergillus</i> and <i>Penicillium</i> spp.	Storage rot Moho negro	Severe in transit
<i>Botrytis allii</i>	Neck rot Podredumbre del cuello	Damage only slight
<i>Rhizoctonia</i> spp., <i>Fusaria</i> spp., and <i>Pythium</i> spp.	Damping-off Mal de almácigo	Severe in all areas
<b>PAPAYA</b> ( <i>Carica papaya</i> )		
<i>Cercospora papayae</i>	Leaf spot Mancha foliar	Little economic loss
<i>Oldum caricae</i>	Powdery mildew Cenicilla	Minor importance
<i>Virus</i>	Papaya bunchy-top virus Virus	Severe in localized areas
<i>Virus</i>	Papaya mosaic virus Mosaico	Minor importance
<b>PEANUT</b> ( <i>Arachis hypogaea</i> )		
<i>Puccinia arachidis</i>	Leaf rust Mancha de hierro	Minor importance
<i>Cercospora personata</i> and <i>C. arachidicola</i> <sup>a</sup>	Leaf spot Mancha foliar	Severe and limiting production
<i>Sclerotium rolfsii</i>	Southern blight Podredumbre del cuello	Severe localized areas
<i>Meloidogyne</i> sp.	Root-knot Hernias radicales	Economic importance undetermined
<i>Pratylenchus</i> sp.	Lesion nematode Raiz negra	Economic importance undetermined
<b>PEPPER</b> ( <i>Capsicum frutescens</i> and <i>C. annuum</i> )		
<i>Cercospora capsici</i>	Frog-eye leaf spot Ojo de sapo	Severe and limiting production
<i>Phoma destructiva</i>	Fruit rot Podricion de la fruta	Moderate damage
<i>Sclerotium rolfsii</i>	Southern blight Podricion del cuello	Severe localized areas
<i>Virus</i>	Undetermined mosaic Mosaico	Minor importance
<i>Meloidogyne</i> sp. <sup>a</sup>	Root-knot Nudo de la raiz	Losses comparatively light
<b>POTATO</b> ( <i>Solanum tuberosum</i> )		
<i>Phytophthora infestans</i> <sup>a</sup>	Late blight Tizon tardio	Severe and limiting production
<i>Alternaria solani</i>	Early blight Tizon temprano	Minor importance
<i>Rhizoctonia solani</i>	Stem rot Mal del tallo	Severe in localized areas
<i>Sclerotium rolfsii</i>	Southern blight Podredumbre del pie	Severe in localized areas
<i>Virus</i> complex	Undetermined Bacterial wilt	Widespread, damaging
<i>Pseudomonas solanacearum</i>	Marchitez	Severe damage
<i>Erwinia carotovora</i>	Soft rot Podredumbre suave	Severe in transit
<i>Meloidogyne incognita</i>	Root-knot Hernias radicales	Losses comparatively light
<i>Pratylenchus penetrans</i>	Lesion nematodes Nematodas	Economic importance undetermined
<b>RICE</b> ( <i>Oryza sativa</i> )		
<i>Leptosphaeria salvinii</i>	Stem rot Mal del tallo	Minor importance
<i>Entyloma oryzae</i> <sup>a</sup>	Leaf smut Carbon de la hoja	Yields not affected
<i>Neovossia barclayana</i> <sup>a</sup>	Kernel smut Anublo del grano	Minor importance
<i>Ustilaginoides virens</i> <sup>a</sup>	False smut Carbon falso	Minor importance
<i>Aspergillus</i> spp. and <i>Penicillium</i> spp.	Kernel discoloration and deterioration Moho del grano	Reduces quality severely
<i>Cercospora oryzae</i> <sup>a</sup>	Narrow brown spot Mancha parda delgada	Minor importance
<i>Helminthosporium oryzae</i> <sup>a</sup>	Brown spot Mancha carmelita	Widespread and damaging
<i>Nigrospora oryzae</i>	Kernel discoloration Mancha del grano	Little economic loss
<i>Pricularia oryzae</i> <sup>a</sup>	Blast Brusome	Widespread with heavy losses
<i>Rhizoctonia</i> spp.	Sheath rot Mancha del tallo	Losses comparatively light
<i>Hoja blanca</i> <sup>a</sup>	Rice stripe virus Mancha blanca rayada (Cuba: "Hoja blanca")	Severe in localized areas, potential danger
<i>Aphelenchoides oryzae</i> <sup>a</sup>	White tip Punta blanca	Minor importance
<b>SESAME</b> ( <i>Sesamum indicum</i> )		
<i>Cercospora sesami</i>	Leaf spot Cercosporiosis	Severe and limiting
<i>Rhizoctonia</i> sp.	Stem blight Chupadera fungosa	Severe in localized areas

Table 1. (concluded)

Host and pathogen	Common name of disease (English, Spanish)	Relative economic importance
<b>SORGHUM</b> ( <i>Sorghum vulgare</i> )		
<i>Sclerospora graminicola</i>	Downy mildew Mildiu	Minor importance
<i>Puccinia purpurea</i>	Leaf rust Roya	Minor importance
<i>Aspergillus</i> spp., <i>Penicillium</i> spp. and <i>Fusarium</i> spp.	Head blight Brusome	Limiting grain production
<i>Colletotrichum falcatum</i>	Anthrachnose Anthrachnosis	Losses comparatively light
<i>Gloeocercospora sorghi</i>	Zonate leaf spot Mancha zonal	Minor importance
<i>Helminthosporium turcicum</i> <sup>a</sup>	Blight Tizon	Widespread and damaging
<b>SUGARCANE</b> ( <i>Saccharum officinarum</i> )		
<i>Leptosphaeria sacchari</i>	Ring spot Mancha circular	Yields not affected
<i>Cercospora longipes</i>	Brown spot Mancha parda	Little economic loss
<i>Colletotrichum falcatum</i>	Red rot Muermo rojo	Widespread damaging
<i>Nigrospora oryzae</i>	Leaf mold Moho de la hoja	Minor importance
<i>Stagonospora sacchari</i>	Leaf scorch Mancha larga	Damage only slight
<i>Xanthomonas vasculorum</i>	Gummosis Gomosis	Severe in localized areas
<i>Virus</i>	Sugarcane streak virus Mal de rayas amarillas	Minor importance
<i>Virus</i>	Mosaic strains A, C, and D Mosaico, A, C, and D	Widespread and damaging
<b>TOBACCO</b> ( <i>Nicotiana tabacum</i> )		
<i>Phytophthora parasitica</i> var. <i>nicotianae</i>	Black shank Base negro	Severe in localized areas
<i>Pythium</i> spp. <sup>b</sup>	Damping-off Mal de almácigo	Widespread and damaging
<i>Alternaria longipes</i> <sup>b</sup>	Brown spot Mancha parda	Minor in importance
<i>Cercospora nicotianae</i> <sup>b</sup>	Frog-eye leaf spot Ojo de sapo	Widespread and damaging
<i>Rhizoctonia solani</i>	Damping-off Mal de almácigo	Severe in localized areas
<i>Sclerotium rolfsii</i> <sup>b</sup>	Southern blight Podricion de la raiz	Severe in localized areas
<i>Bacillus cereus</i> <sup>b</sup>	Frenching Hoja loca	Minor importance
<i>Erwinia aroideae</i> <sup>b</sup>	Hollow stalk Tallo hueco	Minor importance
<i>Pseudomonas solanacearum</i> <sup>b</sup>	Bacterial wilt Marchitez	Severe and limiting
<i>Virus</i> <sup>b</sup>	Tobacco mosaic virus Mosaico	Severe in localized areas
<i>Virus</i> <sup>b</sup>	Leaf curl virus Hoja enrollado	Minor importance
<i>Meloidogyne incognita</i> <sup>b</sup>	Root-knot Hernias radicales	Widespread and severe
<i>Pratylenchus</i> sp. <sup>b</sup>	Lesion nematodes Nematodas	Economic losses undetermined
<i>Helicotylenchus</i> sp. <sup>b</sup>	Spiral nematodes Nematodas	Economic losses undetermined
<i>Trichodorus</i> sp. <sup>b</sup>	Needle nematodes Nematodas	Economic losses undetermined
<b>TOMATO</b> ( <i>Lycopersicon esculentum</i> )		
<i>Phytophthora infestans</i> <sup>a</sup>	Late blight Tizon tardio	Severe in localized areas
<i>Pythium</i> spp.	Damping-off and fruit rot	Severe and widespread
<i>Alternaria solani</i>	Early blight Tizon temprano	Widespread and damaging
<i>Cladosporium fulvum</i>	Leaf mold Moho de la hoja	Severe and widespread
<i>Rhizoctonia solani</i>	Damping-off Mal de almácigo	Severe and widespread
<i>Sclerotium rolfsii</i>	Southern blight Podricion del cuello	Severe in localized areas
<i>Erwinia carotovora</i>	Soft rot Podredumbre suave	Minor importance
<i>Pseudomonas solanacearum</i>	Bacterial wilt Marchitez bacteriana	Severe and limiting production
<i>Virus</i>	Tobacco mosaic virus Mosaico	Losses comparatively light
<i>Virus</i>	Tomato bunchy-top virus	Losses comparatively light
<i>Virus</i>	Undetermined virus complex	Loss undetermined
<i>Meloidogyne incognita</i> <i>acrita</i> <sup>a</sup>	Root-knot Hernias radicales	Widespread and damaging
<b>WATERMELON</b> ( <i>Citrullus vulgaris</i> )		
<i>Pseudoperonospora cubensis</i> <sup>a</sup>	Downy mildew Mildiu	Widespread and damaging
<i>Erysiphe cichoracearum</i>	Powdery mildew Cenicilla	Widespread and damaging
<i>Colletotrichum lagenarium</i>	Anthrachnose Anthrachnosis	Losses comparatively light
<i>Sclerotium rolfsii</i>	Southern blight Podricion del cuello	Severe in localized areas
<i>Meloidogyne</i> sp. <sup>a</sup>	Root-knot Hernias radicales	Widespread, severe
<b>YAM</b> ( <i>Dioscorea alata</i> )		
<i>Cercospora carbonacea</i>	Leaf blotch Cangrena foliar	Severe in localized areas
<i>Diplodia theobromae</i>	Root rot Podricion de la raiz	Little economic loss

<sup>a</sup>Diseases previously reported by Cralley, 1954.<sup>b</sup>Diseases previously reported by Lucas, 1958.

CONTROL OF FUSARIUM STEM ROT OF CARNATIONS:  
I. APPLICATION OF FUNGICIDES TO MOTHER BLOCKS<sup>1</sup>

L. J. Petersen<sup>2</sup>, Ralph Baker, and R. E. Skiver<sup>3</sup>

Summary

Fungicides were sprayed on carnation mother blocks in attempts to control *Fusarium* stem rot of carnations. The manganese salt derivative of pyridinethione (OM 1564) was the most effective. In addition, rooting of carnation cuttings derived from mother blocks sprayed with this material was stimulated significantly after eight applications. OM 1564 compound is not available commercially. Of the compounds available, captan merits recommendation as a spray for carnation mother blocks.

Cuttings used for propagation in the Colorado carnation industry usually are derived from mother blocks. These are a part of a culturing program (6). Attempts to control *Fusarium* stem rot, incited by *Fusarium roseum* f. *cerealis* (Cke.) Snyder & Hans. (5), with conventional sanitation measures such as steaming and disinfestation of tools and soil (1) have not been successful.

In contrast to other pathogens, which are systemic in carnation plants (6), there is no evidence that *F. roseum* f. *cerealis* may be carried over within cuttings (2). As many as 125,000 conidia per cutting of this pathogen, however, have been dislodged from surfaces by washing the cuttings with detergent solution.

The methods currently employed for producing propagative material in mother blocks are conducive to carry-over of inoculum on the surface of cuttings. Mother plants are relatively short and bushy. The lower leaves are shaded and soon die. They may be inundated periodically with nutrient solutions applied by modern watering systems. These factors may contribute to the nutriment of the fungus on the aerial portions of the plants. Further, these watering systems have been observed to splash water and soil particles to a distance of 1 to 3 feet above the bench. Again, the continuous removal of cuttings may afford avenues of entry for the pathogen through wounds.

In conventional operations, carnations are propagated from cuttings in 2 or 3 weeks. Shortly after transplanting there is evidence that some varieties of carnations are resistant (3).

These considerations suggest that fungicidal sprays should be applied to mother blocks, thus insuring a reduction in inoculum during the period when carnations are most susceptible, that is, during propagation.

MATERIALS AND METHODS

Uniform mother blocks were artificially infested by spraying the plants with a suspension containing 100,000 conidia per ml of *F. roseum* f. *cerealis*. The plants were allowed to dry for 24 hours before treatments were applied. Each mother block contained 30 plants (spaced at 8-inch intervals) which received 500 ml of the fungicidal solution or suspension. The compounds and the concentrations at which they were used are listed in Table 1. Colloidal X 77 at 4 ounces/100 gallons of spray solution was used as a wetting agent.

After treatments cuttings were taken from the mother blocks, treated with rooting hormone<sup>4</sup>, and placed in flats for propagation. After a 21-day rooting period the cuttings were rated for disease severity or isolations were made to determine the incidence of infection. The degree of rooting was determined also and, in some cases, cuttings were transplanted in order to observe further the development of symptoms.

RESULTS

Separate mother blocks, containing the varieties Red Gayety and Miller's Yellow, were sprayed each week for 3 weeks with a suspension of conidia of *F. roseum* f. *cerealis*. One day

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<sup>4</sup> Rooting promoter used was Stim-Root (supplied by Plant Products Corp.) containing 98 percent talc, .1 percent indole butyric acid, .25 percent alpha naphthaleneacetic acid, and 1.65 percent tetrachlorobenzoquinone.

Table 1. Materials used, their chemical composition, and name of the supplier.

Material	Concentration (ppm)	Chemical Composition	Manufacturer or Distributor
Captan	1000	N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide	California Spray-Chemical Corp.
Ferbam	1000	ferric dimethyldithio carbamate	E. I. du Pont de Nemours and Co.
Glyodin	1000	2 heptadecyl-2-imidazoline acetate	Carbide and Carbon Chemicals Co.
Panogen 15	27.5	cyano (methylmercuri) guanidine	Morton Chemical Co.
Phaltan	1000	N-trichloromethylthiophthalimide	California Spray-Chemical Corp.
OM 1456	1000	pyridinethione, disulfide derivative	Mathieson Chemical Corp.
OM 1483	1000	pyridinethione, thiourea derivative	Mathieson Chemical Corp.
OM 1564	1000	pyridinethione, manganese salt derivative	Mathieson Chemical Corp.
CP 376	1000	dichloronitro benzene	Monsanto Chemical Co.

Table 2. Number of cuttings from which isolations<sup>a</sup> of *F. roseum* f. *cerealis* were obtained 21 days after the application of various fungicides.

Fungicide <sup>b</sup>	Red Gayety	Miller's Yellow
Captan	0	2
Ferbam	0	4
Glyodin	4	12
Panogen 15	8	14
Phaltan	14	2
Captan and ferbam	4	0
Captan and Glyodin	0	0
Captan and Panogen 15	6	8
Inoculated control	20	16
Non-inoculated control	0	0

<sup>a</sup> Isolations were attempted from 36 cuttings from each variety.<sup>b</sup> Fungicides were applied twice at weekly intervals.Table 3. Development of *Fusarium* stem rot<sup>a</sup> in carnations derived from mother blocks which had been sprayed with various fungicides.

Fungicide <sup>b</sup>	: Number of plants dead <sup>c</sup> :		: Total number of plants with symptoms <sup>c,d</sup> :	
	Red	Miller's	Red	Miller's
	: Gayety	: Yellow	: Gayety	: Yellow
Captan	0	0	1**	2
Ferbam	0	1	14**	17**
Glyodin	12	19	36	36
Panogen 15	1	3	15**	21**
Phaltan	2	0	11**	3**
Captan and ferbam	0	0	13**	5**
Captan and Glyodin	1	0	21*	16*
Captan and Panogen 15	0	0	5**	2**
Inoculated controls	17	29	35	36
Non-inoculated controls	0	0	0*	0

<sup>a</sup> Symptoms noted after 21-day rooting period and 51 days in the nursery bed.<sup>b</sup> Fungicides were applied three times at weekly intervals.<sup>c</sup> There were 36 plants of each variety in each treatment.<sup>d</sup> Single and double asterisks indicate that differences between the numbers of plants with symptoms in treatments as compared with inoculated controls are significant at 0.05 and 0.01 levels, respectively.

Table 4. Symptoms of *Fusarium* stem rot in Red Gayety carnations derived from mother blocks which had been sprayed with various fungicides.<sup>a</sup>

Treatment	Symptoms after 21-day		Number of plants with symptoms after 60 days in nursery beds <sup>b, c</sup>
	rooting period <sup>b, c</sup>		
	Number of plants	Mean length of	
	with lesions	lesions	
		(in mm)	
OM 1483	10	2**	14**
OM 1456	11	2**	24**
OM 1564	5	1**	6**
Captan	15	3**	23**
CP 376	22	6**	48
Panogen 15	10	3**	16**
Inoculated control	39	11	52
Non-inoculated control	6	1**	6**

<sup>a</sup> Fungicides were applied three times at weekly intervals. After each application cuttings were harvested and development of symptoms observed. Thus figures represent the summation of three experiments.

<sup>b</sup> The total number of plants sampled from each treatment was 108. This represents samples of 12 plants replicated three times in each of three experiments.

<sup>c</sup> Single and double asterisks indicate that differences between the numbers of plants with symptoms in treatments as compared with inoculated controls are significant at 0.05 and 0.01 levels, respectively.

Table 5. Degree of rooting of Red Gayety carnations at the end of a 21-day rooting period.

Treatment	Mean rooting index <sup>a, b</sup>	
	Three pre-harvest sprays	Eight pre-harvest sprays
OM 1483	1.6	.9*
OM 1456	1.6	1.1*
OM 1564	2.3	2.5*
Captan	2.0	2.0
Dichloronitrobenzene	1.9	1.0*
Panogen 15	2.0	2.0
Inoculated control	1.1*	.5*
Non-inoculated control	2.2	2.0

<sup>a</sup> Rooting index: 0, no rooting; 1, poor; 2, good; 3, excellent.

<sup>b</sup> Single and double asterisks indicate that differences between the numbers of plants with symptoms in treatments as compared with non-inoculated controls are significant at 0.05 and 0.01 levels, respectively.

after each of these inoculations sprays containing captan, ferbam, Glyodin, Panogen 15, and Phaltan were applied to the mother blocks. Captan was combined also with ferbam, Glyodin and Panogen 15, respectively,

One day after the second application of the fungicides, 36 cuttings of each variety in each treatment were taken from the mother blocks and rooted for 21 days. At the end of this period isolations from the base of each cutting were attempted to determine whether *F. roseum* f. *cerealis* was present. The results of this experiment are recorded in Table 2.

*F. roseum* f. *cerealis* was found in very few cuttings derived from mother blocks sprayed with captan, ferbam, captan and ferbam, or captan and Glyodin.

To test further the potentialities of these compounds, a like number of cuttings divided into three replications was taken 1 day after the third application of fungicides. These were rooted as before, but in this instance the plants were transplanted to separate nursery beds to observe the development of symptoms. Fifty-one days after transplanting, the number of dead plants and plants with distinct lesions characteristic of *Fusarium* stem rot were noted. This experiment was designed to duplicate what might happen under conventional culture, as spread of the pathogen in the beds was possible. The results of this experiment are recorded in Table 3.

Captan appeared to be the best spray material used in these tests for the control of *Fusarium* stem rot. Phytotoxicity, as evidenced by slow root formation, was observed in cuttings treated with Glyodin or Panogen 15.

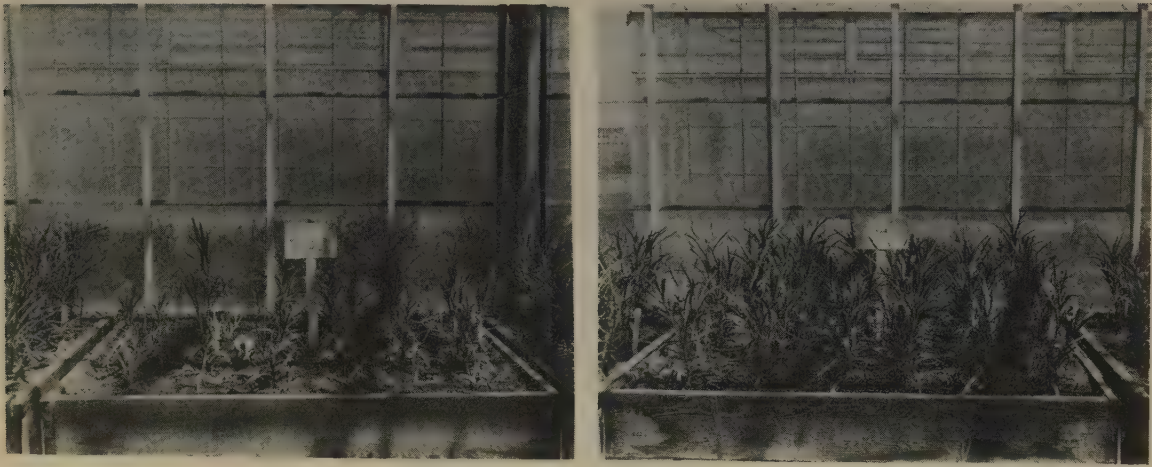


FIGURE 1. Symptoms of *Fusarium* stem rot in plants derived from infested mother blocks after 60 days in the nursery bed. Left -- Plants from non-sprayed infested mother block. Right -- Plants from infested mother block sprayed with OM 1564.

There was evidence in preliminary tests that two applications of OM 1483 not only controlled *Fusarium* stem rot but stimulated rooting of cuttings (4). Thus, further experiments were initiated involving experimental Omadine compounds.

A summation of three consecutive pre-harvest spray experiments involving the variety Red Gayety is recorded in Table 4. Disease indices were recorded at the end of a 21-day rooting period.

OM 1564 was the most effective in controlling *Fusarium* stem rot. There were 50 percent fewer plants with lesions in the OM 1564 treated plots than in the next best treatments (OM 1483 and Panogen 15). In addition, the mean length of lesions for cuttings treated with OM 1564 was 1 mm compared with 11 mm for the inoculated control. At the end of 60 days in the nursery bed, the degree of disease control was even more pronounced. Table 5 and Figure 1 show that cuttings sprayed with OM 1564 compared favorably with the non-inoculated control.

While Panogen 15 was somewhat effective in controlling *Fusarium* stem rot, an undesirable chlorosis developed in the mother plants after the sixth spray application.

Table 5 shows the effects of three and eight successive pre-harvest sprays on rooting of carnations. Rooting was significantly better after eight spray applications with OM 1564 than in the non-inoculated control (Fig. 2). In contrast rooting of cuttings sprayed with OM 1456, OM 1483, and CP 376 was inhibited significantly after eight successive spray applications (Fig. 3). No significant increase or decrease in rooting was observed in any treatment with three pre-harvest sprays except in inoculated controls.

After a few minutes' exposure to either sunlight or fluorescent light, OM 1564 has been observed to change color. The full significance of this phenomenon is not known; however, after exposures to light up to 24 hours, its fungicidal properties against *F. roseum* f. *cerealis* were not impaired in laboratory tests.

#### DISCUSSION

The results of these spray trials indicated that OM 1564 was not only the most effective fungicide tested, but it also stimulated rooting. Moreover, this stimulation was over and above that usually provided by conventional rooting hormones. It is probable, however, that certain concentrations of the experimental material are required for this to occur. Eight applications were required before root stimulation was observed as a result of using OM 1564. In contrast, eight applications of OM 1456 and OM 1483 inhibited rooting. In a preliminary experiment two applications of OM 1483 stimulated rooting.

On the basis of these results, OM 1564 appears to be the most promising fungicide for the control of *Fusarium* stem rot in carnation mother blocks. At present this material is not available commercially. Of the compounds now available, captan would appear to merit recommendation as a spray for carnation mother blocks.

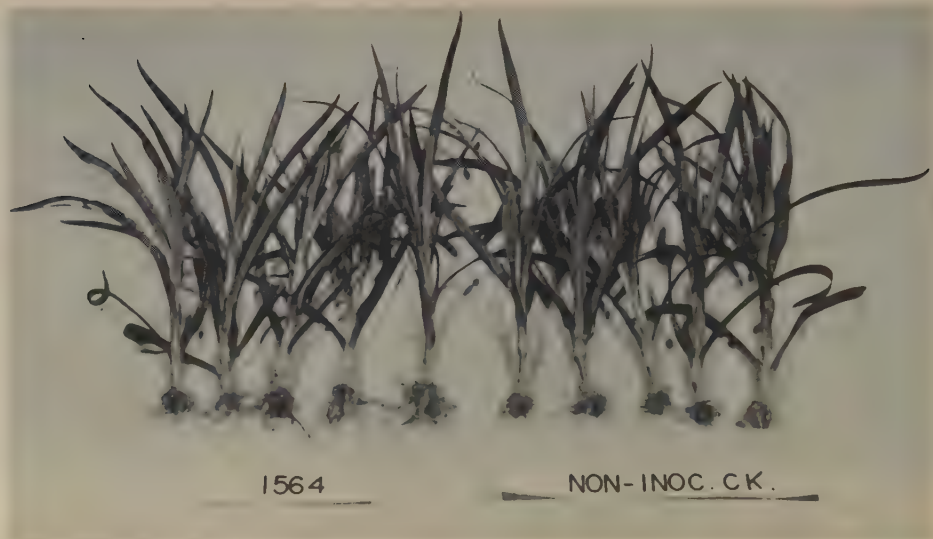


FIGURE 2. Stimulation of rooting after eight applications of OM 1564.

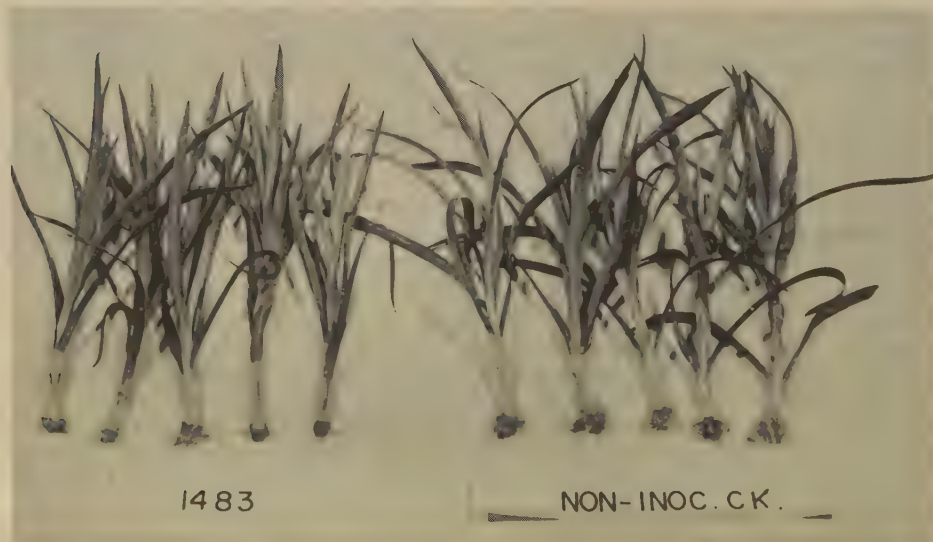


FIGURE 3. Inhibition of rooting after eight applications of OM 1483.

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CONTROL OF FUSARIUM STEM ROT OF CARNATIONS:II. THE USE OF DIPS AND DRENCHES<sup>1</sup>L. J. Petersen<sup>2</sup> and Ralph BakerSummary

Carnation cuttings infested with the conidia of *Fusarium roseum* f. *cerealis* were dipped for 10 minutes in various solutions and suspensions of fungicides in attempts to eradicate this inoculum from cuttings. Results indicated that Pano-drench 4, Panogen Experimental Material WO 4778, and ferbam were effective in control. Drenches were equally effective if they were applied at the beginning of the propagative period.

Combination dips and drenches occasionally gave better control but were slightly phytotoxic.

Carnation cuttings derived from mother blocks in a pathogen-free stock program (3) have been observed to carry externally inoculum of *Fusarium roseum* f. *cerealis* (Cke.) Snyd. & Hans. Research has indicated that fungicidal sprays applied to mother blocks may reduce losses caused by this fungus (2). These experimental results have been substantiated by observational evidence in commercial operations; however, small losses still have occurred sporadically.

Even with the excellent surfactants now available, fungicidal sprays may not cover the entire carnation plant. This may be true especially of leaf axils where inoculum is likely to lodge. Further, in some commercial operations, sprays are not feasible while cuttings are still on the mother plants. Thus, a series of experiments was initiated to investigate the possibility of eradicating inoculum by means of fungicidal dips and drenches.

Preliminary experiments indicated that a solution of sodium hypochlorite containing 0.26 percent available chloride (a 5 percent solution of fresh Clorox) was effective in eradicating the inoculum from infested cuttings (1, 3). Cuttings treated in this manner, however, rooted slowly and were slightly off-color.

MATERIALS AND METHODS

Red Gayety and Miller's Yellow carnation cuttings 6 to 8 inches long were taken from mother blocks. These cuttings were inoculated uniformly by immersing for 5 seconds in a spore suspension containing 100,000 conidia of *F. roseum* f. *cerealis* per ml. Each dip and/or drench treatment was applied to 36 cuttings of each variety in each trial.

In the dip trials inoculated cuttings were immersed and agitated for 10 minutes in test solutions containing the fungicide and 2 drops per gallon of Tween. After the treatments, cuttings were propagated under intermittent mist in steamed Perlite contained in 6-inch pots. Drenches were applied to inoculated cuttings at the beginning of the propagative period at the rate of 1 gallon of solution to 2-3 square feet of Perlite.

At the end of a 21-day propagative period, all the cuttings were removed from the pots, washed and rated for severity of disease and degree of root formation. They were then transplanted to steamed soil in nursery bed plots. After 60 days in these plots the number of dead plants in each treatment was recorded.

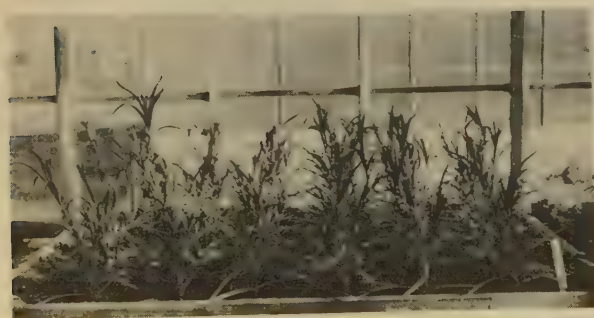
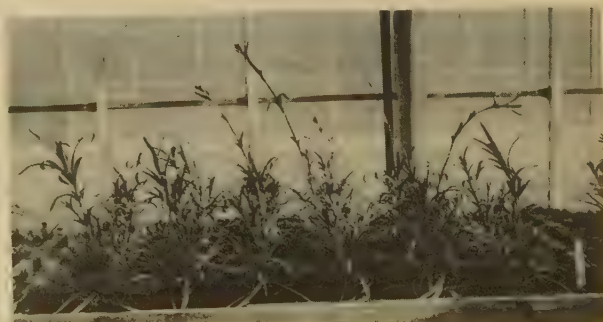
RESULTS AND CONCLUSIONS

The materials listed in Table 1 were screened for their efficacy in eradicating inoculum from cuttings. Most of these were not effective; however, Table 2 records a typical dip and drench test using two of the most promising materials (Pano-drench 4 and WO 4778) from four previous tests, and also Glyodin and ferbam. In this test Pano-drench 4, WO 4778, and ferbam were effective in controlling *Fusarium* stem rot. Figure 1 illustrates the typical condition, after 60 days in nursery beds, of plants which had been treated with Pano-drench 4 and with ferbam.

Glyodin used as a dip was effective in control but rooting was inhibited. Drenches containing this material prevented root initiation.

<sup>1</sup> This work was supported in part by funds from the Colorado Flower Growers Association, Inc. Published with the approval of the Director, Colorado Agricultural Experiment Station as Scientific Series Paper No. 625.

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**INOCULATED CONTROL****NON-INOCULATED CONTROL****PANO-DRENCH 4 DIP****FERBAM DIP****PANO-DRENCH 4 DRENCH****FERBAM DRENCH****PANO-DRENCH 4 DIP+DRENCH****FERBAM DIP+DRENCH**

**FIGURE 1.** Condition after 60 days in the nursery beds of Red Gayety carnations derived from inoculated cuttings treated with dips and/or drenches.

Table 1. Materials used, their chemical composition, and name of the supplier.

Material	Concentration of active ingredient (ppm)	Chemical Composition	Manufacturer or Distributor
OM 1084	50 and 100	---	Olin Mathieson Chemical Corp.
Hyamine 2389	2000	Methyl dodecyl benzyl trimethyl and methyl dodecyl xylylene bis trimethyl ammonium chlorides	Rohm and Haas Co.
-----	1000	Potassium permanganate	---
Captan	1000	N-(trichloromethylthio)-4-cyclohexine-1,2-dicarboximide	California Spray-Chemical Corp.
Rimocidin	1000	---	Chas. Pfizer and Co., Inc.
Catechol		dihydroxy benzene (o)	
-----	1000	2,5 dimethyl-quinone	Monsanto Chemical Co.
-----	1000	p benzoquinone	Monsanto Chemical Co.
-----	1000	2,6-dichloro-quinone	Monsanto Chemical Co.
WO 4778	3.0	---	Morton Chemical Co.
Pano-drench 4	2.6	cyano (methyl-mercuri) guanidine	Morton Chemical Co.
Ferbam	1000	ferric dimethyldithio carbamate	E. I. du Pont de Nemours and Co.
Glyodin	1000	2 heptadecyl-2-imidazoline acetate	Carbon Chemicals Corp.

Table 2. Severity of disease and rooting indices after treating two varieties of carnation cuttings<sup>a</sup> with various fungicidal solutions.

Material	Treatment	Concentration of active ingredient (ppm)	Number of plants with lesions after		Rooting indices at end of 21-		Number of plants dead after 60	
			21-day rooting		day rooting		days in	
			period		period <sup>b</sup>		nursery beds	
			: Miller's	: Red Sim:	: Red	: Miller's:	: Red	: Miller's
			: Red Sim:	: Yellow	: Gayety:	: Yellow	: Gayety:	: Yellow
Ferbam	Dip	1000	2	0	2.6	3.0	4	2
	Dip + drench	1000	0	0	2.2	2.3	3	3
	Drench	1000	3	0	1.9	1.3	3	5
Pano-drench 4	Dip	2.6	0	3	2.8	2.3	0	4
	Dip + drench	2.6	0	1	2.3	1.8	1	1
	Drench	2.6	0	1	2.0	2.0	0	1
WO 4778	Dip	3.0	1	1	2.4	2.7	2	2
	Dip + drench	3.0	1	1	2.3	1.0	1	1
	Drench	3.0	2	3	2.0	1.3	4	5
Glyodin	Dip	1000	3	6	1.3	1.3	3	6
	Dip + drench	1000	(Phytotoxic, no further evaluation)					
	Drench	1000	(Phytotoxic, no further evaluation)					
---	Inoc. Control	---	36	35	0	0.1	27	33
---	Non-inoc. Control	---	0	3	2.3	2.2	0	0

<sup>a</sup> Thirty-six cuttings of each variety were included in each treatment.<sup>b</sup> Rooting index: 0, no rooting; 1, poor; 2, good; 3, excellent.

Slight phytotoxicity, as evidenced by inhibition of rooting, was noted in some instances. Cuttings drenched with ferbam did not root as rapidly as the non-inoculated controls (Table 2). In some trials the combination dip and drench with either Pano-drench 4 or WO 4778 resulted in a slight reduction in the rooting indices (for example, Table 1, WO 4778); however, this phytotoxicity was not apparent 60 days after transplanting rooted cuttings into the nursery bed. In other experiments it was established that Pano-drench 4 and WO 4778 were excessively phytotoxic at concentrations exceeding 5 ppm active ingredient.

From the standpoint of minimum toxicity and maximum disease control, dips or drenches using Pano-drench 4, WO 4778, or ferbam would merit recommendation. In mother block operations, however, sprays have been just as effective in control (2) and are easier to apply. Thus the use of dips and drenches for the control of *Fusarium* stem rot might well be confined to occasions when sprays are not feasible, for example, when cuttings for propagation are derived from cut flower stems.

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#### BRIEF NOTE

##### DITYLENCHUS DESTRUCTOR FROM GRASS, DAHLIA AND GLADIOLUS INFECTING POTATO TUBERS

By Grover C. Smart, Jr.

Three collections of nematodes morphologically indistinguishable from *Ditylenchus destructor* Thorne were obtained from the following sources: 1) a lawn of St. Augustine grass (*Stenotaphrum secundatum* (Walt.) Kuntze) in Arkansas; 2) dahlia tuberous roots (*Dahlia* sp.) submitted for verification of identity of the nemas by Dr. H. J. Jensen<sup>1</sup>; 3) decaying roots of a gladiolus bulb (*Gladiolus* sp.) of unknown origin. The nemas from the latter source were probably feeding on fungi rather than on the roots.

The three collections were transferred aseptically to a fungus culture of *Torula* sp. and to tobacco callus tissue. Large populations of each isolate developed in 70 to 90 days.

Approximately 6000 nemas of each isolate were used to inoculate 50-day-old potato plants of the Sebago variety growing in 8-inch pots. An isolate of *Ditylenchus destructor* from Wisconsin known to be pathogenic to potatoes and an uninoculated control were also included for comparison. Three replicates were used. Inoculations were made by digging three small holes about 3 inches deep in each pot and adding a prepared water suspension of the nemas. The holes were immediately covered with soil and water added as needed. Soil temperature in the greenhouse was maintained at about 73° to 75° F.

Tubers were examined after 2 months and all isolates were pathogenic. Symptoms were confined to the tubers and were typical of those found in the field and those obtained in the greenhouse with known pathogenic isolates. The uninoculated control showed no infection. Nemas from each isolate taken directly from tubers were found to be morphologically identical with their parent cultures.

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<sup>1</sup> Jensen, H. J., Harriet R. Smithson, and L. B. Loring. 1958. Potato-rot nematode, *Ditylenchus destructor* Thorne 1945, found in Dahlia roots. Plant Disease Repr. 42: 1357-1359.

BOOK REVIEW

"PLANT PATHOLOGY: PROBLEMS AND PROGRESS 1908-1958." The University of Wisconsin Press, Madison, Wis. xix + 588 pages. 1959. Price \$8.50.

This book is the product of 51 authors, five editors, and the American Phytopathological Society. It is, in fact, the Society's Golden Jubilee volume. It collects the major addresses and symposium papers presented by some of the world's most noted scientists before the 50th anniversary meeting of the Society at Bloomington, Indiana in August 1958.

Thus, "Plant Pathology" is a broad treatment of the science. It contains something to interest every biologist, not only for plant pathologists, but also for specialists in such related sciences as genetics, physiology, and biochemistry; and indeed, there is much for young students of biology.

The book has an important place in the literature of plant pathology and of other biological sciences. Carefully, and to a degree philosophically, it develops the history of plant pathology and projects its future.

The book is in ten parts. Each part is devoted to a major division of the science. Most of the book -- parts two through ten -- contains the papers presented before the nine symposia of the Anniversary meeting. There are six papers on physiology, seven on genetics, seven on fungicides and the chemistry of fungicides, six on fungi, five on nematology, eight on the structure and the multiplication of viruses, and five on epidemiology.

The authors of these papers are among the most distinguished in their own fields. For example, G. J. M. van der Kerk, of the Institute for Organic Chemistry, The Netherlands, contributes a paper on the "Chemical Structure and Fungicidal Activity of Dithiocarbamic Acid Derivatives," and Gerhard Schramm, of the Max Planck Institute for Virus Research, Germany, discusses "The Role of the Nucleic Acid in the Infection with Tobacco Mosaic Virus."

It is significant that these two contributors came from foreign lands. Twenty-one of the 44 papers included in the symposia were presented by scientists brought here from England, Australia, South Africa, Germany, The Netherlands, Honduras, Mexico, and Canada.

Part One of the Golden Jubilee volume contains the seven major addresses concerned with the history and development of plant pathology. Again, the prestige of the authors and the quality of their papers are outstanding. E. C. Stakman of the University of Minnesota relates plant pathology to the world's scientific and social development; John A. Stevenson of the United States Department of Agriculture traces the development of the science in North America; and J. G. Harrar of the Rockefeller Foundation discusses an international approach to the study and control of plant diseases. Other contributors to this section are S. E. A. McCallan and George L. McNew of the Boyce Thompson Institute for Plant Research, J. C. Walker of the University of Wisconsin, and James G. Horsfall of the Connecticut Agricultural Experiment Station.

All in all, "Plant Pathology" reflects much credit on the various committees of the Society that were responsible for the presentation and publication of these papers, the contents of which point to the significant role of biological sciences in the future of mankind.

The editors and publisher should be complimented on the production of this book which not only is very readable but also is handsomely bound in gold.

